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- (56) References cited:

EP-A- 0 121 976 EP-A- 0 128 041 EP-A- 0 148 155 EP-A- 0 212 474 WO-A-85/04173 US-A- 4 455 256 US-A- 4 563 350 US-A- 4 619 989

- PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA, vol. 81, January 1984, Washington, DC (US); URIST, pp. 371-375
- SCIENCE, vol. 220, 13 May 1983, Washington, DC (US); URIST, pp. 680-686
- PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA, vol. 80, November 1983, Washington, DC (US); SAMPATH et al., pp. 6591-6595
- PROCEEDINGS OF THE NATL. ACADEMY OF SCIENCES USA, vol. 78, November 1981, Washington, DC (US); SUGGS et al., pp. 6613-6617

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Description

The present invention relates to novel proteins, processes for obtaining them and genes encoding them. These proteins are capable of inducing cartilage and bone formation.

Background

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Bone is a highly specialized tissue characterized by an extensive matrix structure formed of fibrous bundles of the protein collagen, and proteoglycans, noncollagenous proteins, lipids and acidic proteins. The processes of bone formation and renewal/repair of bone tissue, which occur continuously throughout life, are performed by specialized cells. Normal embryonic long bone development is preceded by formation of a cartilage model. Bone growth is presumably mediated by "osteoblasts" (bone-forming cells), while remodeling of bone is apparently accomplished by the joint activities of bone-resorbing cells, called "osteoclasts" and osteoblasts. A variety of osteogenic, cartilage-inducing and bone inducing factors have been described. See, e.g. European patent applications 148,155 and 169,016 for discussions thereof.

Brief Description of the Invention

The present invention provides novel proteins in purified form and genes encoding them. Specifically, two of the novel proteins are designated BMP-2 Class I (or BMP-2), and BMP-2 Class II (or BMP-4) wherein BMP is bone morphogenic protein. These proteins are characterized by peptide sequences the same as or substantially homologous to amino acid sequences illustrated in Tables II, III and IV below. They are capable of inducing bone formation at a predetermined site. These bone inductive factors are further characterized by biochemical and biological characteristics including activity at a concentration of 10 to 1000ng/gram of bone in an in vivo rat bone formation assay described below. Proteins of this invention may be encoded by the DNA sequences depicted in the Tables or by sequences capable of hybridizing thereto and coding for polypeptides with bone growth factor biological properties or other variously modified sequences demonstrating such properties.

One of the proteins of the invention is designated BMP-2 Class I (or BMP-2). It is characterized by at least a portion of a peptide sequence the same or substantially the same as that of amino acid #1 through amino acid #396 of Table III which represents the cDNA hBMP-2 Class I. This peptide sequence is encoded by the same or substantially the same DNA sequence, as depicted in nucleotide #356 through nucleotide #1543 of Table III. The human peptide sequence identified in Table III is 396 amino acids in length. hBMP-2 or related bone inductive proteins may also be characterized by at least a portion of this peptide sequence. hBMP-2 Class I is further characterized by the ability to induce bone formation.

The homologous bovine bone inductive protein of the invention designated bBMP-2 Class I (or bBMP-2), has a DNA sequence identified in Table II below which represents the genomic sequence. This bovine DNA sequence has a prospective 129 amino acid coding sequence followed by approximately 205 nucleotides (a presumptive 3' non-coding sequence). bBMP-2, Class I is further characterized by the ability to induce bone formation. A further bone inductive protein composition of the invention is designated BMP-2 Class II or BMP-4. The human protein hBMP-2 Class II (or hBMP-4) is characterized by at least a portion of the same or substantially the same peptide sequence between amido acid #1 through amino acid #408 of Table IV, which represents the cDNA of hBMP-2 Class II. This peptide sequence is encoded by at least a portion of the same or substantially the same DNA sequence as depicted in nucleotide #403 through nucleotide #1626 of Table IV. This factor is further characterized by the ability to induce bone formation.

Another aspect of the invention provides pharmaceutical compositions containing a therapeutically effective amount of one or more bone growth factor polypeptides according to the invention in a pharmaceutically acceptable vehicle. These compositions may further include other therapeutically useful agents. They may also include an appropriate matrix for delivering the proteins to the site of the bone defect and for providing a structure for bone growth. These compositions may be employed in methods for treating a number of bone defects and periodontal disease. These methods, according to the invention, entail administering to a patient needing such bone formation an effective amount of at least one of the novel proteins BMP-2 Class I and BMP-2 Class-II as described herein.

Still a further aspect of the invention are DNA sequences coding on expression for a human or bovine polypeptide having the ability to induce bone formation. Such sequences include the sequence of nucleotides in a 5' to 3' direction illustrated in Tables II, III and IV. Alternatively, a DNA sequence which hybridizes under stringent conditions with the DNA sequences of Tables II, III and IV or a DNA sequence which hybridizes under non-stringent conditions with the illustrated DNA sequences and which codes on expression for a protein having at least one bone growth factor biological property are included in the present invention. Finally, allelic or other variations of the sequences of Tables II, III and IV, whether such nucleotide changes result in changes in the peptide sequence or not, are also included in the present

invention.

Still a further aspect of the invention is a vector containing a DNA sequence as described above in operative association with an expression control sequence. Such vector may be employed in a novel process for producing a bone growth factor polypeptide in which a cell line transformed with a DNA sequence encoding expression of a bone growth factor polypeptide in operative association with an expression control sequence therefor, is cultured. This claimed process may employ a number of known cells as host cells for expression of the polypeptide. Presently preferred cell lines are mammalian cell lines and bacterial cells.

Other aspects and advantages of the present invention will be apparent upon consideration of the following detailed description and preferred embodiments thereof.

Detailed Description of the Invention

The proteins of the present invention are characterized by amino acid sequences or portions thereof the same as or substantially homologous to the sequences shown in Tables II, III and IV. These proteins are also characterized by the ability to induce bone formation.

The bone growth factors provided herein also include factors encoded by the sequences similar to those of Tables II, III and IV, but into which modifications are naturally provided (e.g. allelic variations in the nucleotide sequence which may result in amino acid changes in the polypeptide) or deliberately engineered. For example, synthetic polypeptides may wholly or partially duplicate continuous sequences of the amino acid residues of Tables II, III and IV. These sequences, by virtue of sharing primary, secondary, or tertiary structural and conformational characteristics with bone growth factor polypeptides of Tables II, III and IV may possess bone growth factor biological properties in common therewith. Thus, they may be employed as biologically active substitutes for naturally-occurring bone growth factor polypeptides in therapeutic processes.

Other specific mutations of the sequences of the bone growth factors described herein involve modifications of one or both of the glycosylation sites. The absence of glycosylation or only partial glycosylation results from amino acid substitution or deletion at one or both of the asparagine-linked glycosylation recognition sites present in the sequences of the bone growth factors shown in Tables II, III and IV. The asparagine-linked glycosylation recognition sites comprise tripeptide sequences which are specifically recognized by appropriate cellular glycosylation enzymes. These tripeptide sequences are either asparagine-X-threonine or asparagine-X-serine, where X is usually any amino acid. A variety of amino acid substitutions or deletions at one or both of the first or third amino acid positions of a glycosylation recognition site (and/or amino acid deletion at the second postion) results in non-glycosylation at the modified tripeptide sequence.

The present invention also encompasses the novel DNA sequences, free of association with DNA sequences encoding other proteinaceous materials, and coding on expression for bone growth factors. These DNA sequences include those depicted in Tables II, III and IV in a 5' to 3' direction and those sequences which hybridize under stringent hybridization conditions [see, T. Maniatis et al, Molecular Cloning (A Laboratory Manual), Cold Spring Harbor Laboratory (1982), pages 387 to 389] to the DNA sequences of Tables II, III and IV.

DNA sequences which hybridize to the sequences of Tables II, III and IV under relaxed hybridization conditions and which code on expression for bone growth factors having bone growth factor biological properties also encode bone growth factors of the invention. For example, a DNA sequence which shares regions of significant homology, e. g., sites of glycosylation or disulfide linkages, with the sequences of Tables II, III and IV and encodes a bone growth factor having one or more bone growth factor biological properties clearly encodes a member of this novel family of growth factors, even if such a DNA sequence would not stringently hybridize to the sequence of Tables II, III and IV.

Similarly, DNA sequences which code for bone growth factor polypeptides coded for by the sequences of Tables II, III and IV, but which differ in codon sequence due to the degeneracies of the genetic code or allelic variations (naturally-occurring base changes in the species population which may or may not result in an amino acid change) also encode the novel growth factors described herein. Variations in the DNA sequences of Tables II, III and IV which are caused by point mutations or by induced modifications to enhance the activity, half-life or production of the polypeptides encoded thereby are also encompassed in the invention.

Another aspect of the present invention provides a novel method for producing the novel osteoinductive factors. The method of the present invention involves culturing a suitable cell or cell line, which has been transformed with a DNA sequence coding on expression for a novel bone growth factor polypeptide of the invention, under the control of known regulatory sequences. Suitable cells or cell lines may be mammalian cells, such as Chinese hamster ovary (CHo) cells. The selection of suitable mammalian host cells and methods for transformation, culture, amplification, screening and product production and purification are known in the art. See, e.g., Gething and Sambrook, Nature, 293: 620-625 (1981), or alternatively, Kaufman et al, Mol. Cell. Biol., 5(7):1750-1759 (1985) or Howley et al, U.S. Patent 4,419,446. Another suitable mammalian cell line, which is described in the accompanying examples, is the monkey COS-1 cell line. A similarly useful mammalian cell line is the CV-1 cell line.

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Bacterial cells are suitable hosts. For example, the various strains of E. <u>coli</u> (e.g., HB101, MC1061) are well-known as host cells in the field of biotechnology. Various strains of <u>B</u>. <u>subtilis</u>, <u>Pseudomonas</u>, other bacilli and the like may also be employed in this method.

Many strains of yeast cells known to those skilled in the art are also available as host cells for expression of the polypeptides of the present invention. Additionally, where desired, insect cells may be utilized as host cells in the method of the present invention. See, e.g. Miller et al, <u>Genetic Engineering</u>, <u>8</u>:277-298 (Plenum Press 1986) and references cited therein.

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Another aspect of the present invention provides vectors for use in the method of expression of these novel osteoinductive polypeptides. Preferably the vectors contain the full novel DNA sequences described above which code for
the novel factors of the invention. Additionally the vectors also contain appropriate expression control sequences permitting expression of the bone inductive protein sequences. Alternatively, vectors incorporating modified sequences
as described above are also embodiments of the present invention and useful in the production of the bone inductive
proteins. The vectors may be employed in the method of transforming cell lines and contain selected regulatory sequences in operative association with the DNA coding sequences of the invention which are capable of directing the
replication and expression thereof in selected host cells. Useful regulatory sequences for such vectors are known to
one of skill in the art and may be selected depending upon the selected host cells. Such selection is routine and does
not form part of the present invention.

A protein of the present invention, which induces bone growth in circumstances where bone is not normally formed, has application in the healing of bone fractures. An osteogenic preparation employing one or more of the proteins of the invention may have prophylactic use in closed as well as open fracture reduction and also in the improved fixation of artificial joints. De novo bone formation induced by an osteogenic agent contributes to the repair of congenital, trauma induced, or oncologic resection induced craniofacial defects, and also is useful in cosmetic plastic surgery. An osteogenic factor of the invention may be valuable in the treatment of periodontal disease, and in other tooth repair processes. Such agents may provide an environment to attract bone-forming cells, stimulate growth of bone-forming cells or induce differentiation of progenitors of bone-forming cells. Of course, the proteins of the invention may have other therapeutic uses.

A further aspect of the invention is a therapeutic method and composition for repairing fractures and other conditions related to bone defects or periodontal diseases. Such a composition comprises a therapeutically effective amount of at least one of the bone inductive factor proteins of the invention. The bone inductive factors according to the present invention may be present in a therapeutic composition in admixture with a pharmaceutically acceptable vehicle or matrix. Further therapeutic methods and compositions of the invention comprise a therapeutic amount of a bone inductive factor of the invention with a therapeutic amount of at least one of the other bone inductive factors of the invention. Additionally, the proteins according to the present invention or a combination of the proteins of the present invention may be co-administered with one or more different osteoinductive factors with which they may interact. Further, the bone inductive proteins may be combined with other agents beneficial to the treatment of the bone defect in question. Such agents include, but are not limited to various growth factors. The preparation of such physiologically acceptable protein compositions, having due regard to pH, isotonicity, stability and the like, is within the skill of the art.

In particular, BMP-2 Class I may be used individually in a pharmaceutical composition. BMP-2 Class I may also be used in combination with one or more of the other proteins of the invention. BMP-2 Class I may be combined with BMP-2 Class II. It may also be combined with BMP-3. Further BMP-2 Class I may be combined with BMP-2 Class II and BMP-3.

BMP-2 Class II may be used individually in pharmaceutical composition. In addition, it may be used in combination with other proteins as identified above. Further it may be used in combination with BMP-3.

The therapeutic method includes locally administering the composition as an implant or device. When administered, the therapeutic composition for use in this invention is, of course, in a pyrogen-free, physiologically acceptable form. Further, the composition may desirably be encapsulated or injected in a viscous form for delivery to the site of bone damage. Preferably, the bone growth inductive factor composition would include a matrix capable of delivering the bone inductive factor to the site of bone damage, providing a structure for the developing bone and cartilage and optimally capable of being resorbed into the body. Such matrices may be formed of other materials presently in use for other implanted medical applications.

The choice of material is based on, for example, biocompatibility, biodegradability, mechanical properties, cosmetic appearance and interface properties. Similarly, the application of the osteoinductive factors will define the appropriate formulation. Potential matrices for the osteoinductive, factors may be biodegradable and chemically defined, such as, but not limited to calcium sulfate, tricalciumphosphate, hydroxyapatite, polylactic acid, polyanhydrides; biodegradable and biologically well defined, such as bone or dermal collagen, other pure proteins or extracellular matrix components; nonbiodegradable and chemically defined, such as sintered hydroxyapatite, bioglass, aluminates, or other ceramics; or combinations of any of the above mentioned types of material, such as polylactic acid and hydroxyapatite or collagen and tricalciumphosphate. The bioceramics might also be altered in composition, such as in calcium-aluminate-phos-

phate and processing to alter for example, pore size, particle size, particle shape, and biodegradability.

The dosage regimen will be determined by the attending physician considering various factors which modify the action of such a growth factor, e.g. amount of bone weight desired to be formed, the site of bone damage, the condition of the damaged bone, the patient's age, sex, and diet, the severity of any infection, time of administration and other clinical factors. The dosage may vary with the type of matrix used in the reconstitution and the composition of BMP's. The addition of other known growth factors, such as IGF 1 (insulin like growth factor 1), to the final composition, may also effect the dosage. Generally, the dosage regimen should be in the range of approximately 10 to 10⁶ nanograms of protein per gram of bone weight desired. Progress can be monitored by periodic assessment of bone growth and/ or repair, e.g. x-rays. Such therapeutic compositions are also presently valuable for veterinary applications due to the lack of species specificity in bone inductive factors. Particularly domestic animals and thoroughbred horses in addition to humans are desired patients for such treatment with the bone inductive factors of the present invention.

The following examples illustrate practice of the present invention in recovering and characterizing the bovine proteins and employing them to recover the human proteins, obtaining the human proteins and in expressing the proteins via recombinant techniques.

EXAMPLE I

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Isolation of Bovine Bone Inductive Factor

Ground bovine bone powder (20-120 mesh, Helitrex) is prepared according to the procedures of M. R. Urist et al., Proc. Natl Acad. Sci USA, 70:3511 (1973) with elimination of some extraction steps as identified below. Ten kgs of the ground powder is demineralized in succesive changes of 0.6N HCl at 4°C over a 48 hour period with vigorous stirring. The resulting suspension is extracted for 16 hours at 4°C with 50 liters of 2M CaCl₂ and 10mM ethylenediamine-tetraacetic acid [EDTA], and followed by extraction for 4 hours in 50 liters of 0.5M EDTA. The residue is washed three times with distilled water before its resuspension in 20 liters of 4M guanidine hydrochloride [GuCl], 20mM Tris (pH 7.4), 1 mM N-ethylmaleimide, 1mM iodoacetamide, 1mM phenylmethylsulfonyl fluoride as described in Clin. Orthop. Rel. Res., 171: 213 (1982). After 16 to 20 hours the supernatant is removed and replaced with another 10 liters of GuCl buffer. The residue is extracted for another 24 hours.

The crude GuCl extracts are combined, concentrated approximately 20 times on a Pellicon apparatus with a 10,000 molecular weight cut-off membrane, and then dialyzed in 50mM Tris, 0.1M NaCl, 6M urea (pH7.2), the starting buffer for the first column. After extensive dialysis the protein is loaded on a 4 liter DEAE cellulose column and the unbound fractions are collected.

The unbound fractions are concentrated and dialyzed against 50mM NaAc, 50mM NaCl (pH 4.6) in 6M urea. The unbound fractions are applied to a carboxymethyl cellulose column. Protein not bound to the column is removed by extensive washing with starting buffer, and the bone inductive factor containing material desorbed from the column by 50mM NaAc, 0.25mM NaCl, 6M urea (pH 4.6). The protein from this step elution is concentrated 20- to 40- fold, then diluted 5 times with 80mM KPO₄, 6M urea (pH6.0). The pH of the solution is adjusted to 6.0 with 500mM K₂HPO₄. The sample is applied to an hydroxylapatite column (LKB) equilibrated in 80mM KPO₄, 6M urea (pH6.0) and all unbound protein is removed by washing the column with the same buffer. Bone inductive factor activity is eluted with 100mM KPO₄ (pH7.4) and 6M urea.

The protein is concentrated approximately 10 times, and solid NaCl added to a final concentration of 0.15M. This material is applied to a heparin - Sepharose column equilibrated in 50mM KPO₄, 150mM NaCl, 6M urea (pH7.4). After extensive washing of the column with starting buffer, a protein with bone inductive factor activity is eluted by 50mM KPO₄, 700mM NaCl, 6M urea (pH7.4). This fraction is concentrated to a minimum volume, and 0.4ml aliquots are applied to Superose 6 and Superose 12 columns connected in series, equilibrated with 4M GuCl, 20mM Tris (pH7.2) and the columns developed at a flow rate of 0.25ml/min. The protein demonstrating bone inductive factor activity has a relative migration corresponding to approximately 30,000 dalton protein.

The above fractions are pooled, dialyzed against 50mM NaAc, 6M urea (pH4.6), and applied to a Pharmacia MonoS HR column. The column is developed with a gradient to 1.0M NaCl, 50mM NaAc, 6M urea (pH4.6). Active fractions are pooled and brought to pH3.0 with 10% trifluoroacetic acid (TFA). The material is applied to a 0.46 x 25cm Vydac C4 column in 0.1% TFA and the column developed with a gradient to 90% acetonitrile, 0.1% TFA (31.5% acetonitrile, 0.1% TFA to 49.5% acetonitrile, 0.1% TFA in 60 minutes at Iml per minute). Active material is eluted at approximately 40-44% acetonitrile. Aliquots of the appropriate fractions are iodinated by one of the following methods: P. J. McConahey et al, Int. Arch. Allergy, 29:185-189 (1966); A. E. Bolton et al, Biochem J., 133:529 (1973); and D. F. Bowen-Pope, J. Biol. Chem., 237:5161 (1982). The iodinated proteins present in these fractions are analyzed by SDS gel electrophoresis and urea Triton X 100 isoelectric focusing. At this stage, the bone inductive factor is estimated to be approximately 10-50% pure.

EXAMPLE II

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Characterization of Bovine Bone Inductive Factor

A. Molecular Weight

Approximately 20ug protein from Example I is lyophilized and redissolved in 1X SDS sample buffer. After 15 minutes of heating at 37°C, the sample is applied to a 15% SDS polyacrylamide gel and then electrophoresed with cooling. The molecular weight is determined relative to prestained molecular weight standards (Bethesda Research Labs). Immediately after completion, the gel lane containing bone inductive factor is sliced into 0.3cm pieces. Each piece is mashed and 1.4ml of 0.1% SDS is added. The samples are shaken gently overnight at room temperature to elute the protein. Each gel slice is desalted to prevent interference in the biological assay. The supernatant from each sample is acidified to pH 3.0 with 10% TFA, filtered through a 0.45 micron membrane and loaded on a 0.46cm x 5cm C4 Vydac column developed with a gradient of 0.1% TFA to 0.1% TFA, 90% CH₃CN. The appropriate bone inductive factor containing fractions are pooled and reconstituted with 20mg rat matrix. In this gel system, the majority of bone inductive factor fractions have the mobility of a protein having a molecular weight of approximately 28,000 - 30,000 daltons.

B. Isoelectric Focusing

The isoelectric point of bone inductive factor activity is determined in a denaturing isoelectric focusing system. The Triton X100 urea gel system (Hoeffer Scientific) is modified as follows: 1) 40% of the ampholytes used are Servalyte 3/10; 60% are Servalyte 7-9. 2) The catholyte used is 40mM NaOH. Approximately 20ug of protein from Example I is lyophilized, dissolved in sample buffer and applied to the isoelectrofocusing gel. The gel is run at 20 watts, 10°C for approximately 3 hours. At completion the lane containing bone inductive factor is sliced into 0.5 cm slices. Each piece is mashed in 1.0ml 6M urea, 5mM Tris (pH 7.8) and the samples agitated at room temperature. The samples are acidified, filtered, desalted and assayed as described above. The major portion of activity as determined in the assay described in Example III migrates in a manner consistent with a pl of 8.8 - 9.2.

C. Subunit Characterization

The subunit composition of bone inductive factor is also determined. Pure bone inductive factor is isolated from a preparative 15% SDS gel as described above. A portion of the sample is then reduced with 5mM DTT in sample buffer and re-electrophoresed on a 15% SDS gel. The approximately 30kd protein yields two major bands at approximately 20kd and 18kd, as well as a minor band at 30kd. The broadness of the two bands indicates heterogeneity caused most probably by glycosylation, other post translational modification, proteolytic degradation or carbamylation.

EXAMPLE III

Biological Activity of Bone Inductive Factor

A rat bone formation assay according to the general procedure of Sampath and Reddi, <u>Proc. Natl. Acad. Sci. U. S.A.</u>, 80:6591-6595 (1983) is used to evaluate the osteogenic activity of the bovine bone inductive factor of the present invention obtained in Example I. This assay can also be used to evaluate bone inductive factors of other species. The ethanol precipitation step is replaced by dialyzing the fraction to be assayed against water. The solution or suspension is then redissolved in a volatile solvent, e.g. 0.1 - 0.2 % TFA, and the resulting solution added to 20mg of rat matrix. This material is frozen and lyophilized and the resulting powder enclosed in #5 gelatin capsules. The capsules are implanted subcutaneously in the abdominal thoracic area of 21 - 49 day old male long Evans rats. The implants are removed after 7 - 14 days. Half of each implant is used for alkaline phosphatase analysis [See, A. H. Reddi et al., <u>Proc. Natl. Acad. Sci.</u>, 69:1601 (1972)] and half is fixed and processed for histological analysis. Routinely, 1µm glycolmeth-acrylate sections are stained with Von Kossa and acid fuchsin to detect new bone mineral. Alkaline phosphatase, an enzyme produced by chondroblasts and osteoblasts in the process of matrix formation, is also measured. New cartilage and bone formation often correlates with alkaline phosphatase levels. Table I below illustrates the dose response of the rat matrix samples including a control not treated with bone inductive factor.

TABLE 1

Protein* Implanted μg	Cartilage	Alk. Phos.u/l
7.5	2	Not done
2.5	3	445.7
0.83	3	77.4
0.28	0	32.5
0.00	0	31.0

*At this stage the bone inductive factor is approximately 10-15% pure.

The bone or cartilage formed is physically confined to the space occupied by the matrix. Samples are also analyzed by SDS gel electrophoresis and isoelectric focusing as described above, followed by autoradiography. Analysis reveals a correlation of activity with protein bands at 28 - 30kd and a pl 9.0. An extinction coefficient of 1 OD/mg-cm is used as an estimate for protein and approximating the purity of bone inductive factor in a particular fraction. In the <u>in vivo</u> rat bone formation assays on dilutions as described above, the protein is active <u>in vivo</u> at 10 to 200ng protein/gram bone to probably greater than 1µg protein/gram bone.

EXAMPLE IV

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Bovine Bone Inductive Factor Protein Composition

The protein composition of Example IIA of molecular weight 28 - 30kd is reduced as described in Example IIC and digested with trypsin. Eight tryptic fragments are isolated by standard procedures having the following amino acid sequences:

Fragment 1: AAFLGDIALDEEDLG

Fragment 2: A F Q V Q Q A A D L

Fragment 3: N Y Q D M V V E G

Fragment 4: S T P A Q D V S R

Fragment 5: NQEALR

Fragment 6: L S E P D P S H T L E E

Fragment 7: F D A Y Y

Fragment 8: LKPSN?ATIQSIVE

A less highly purified preparation of protein from bovine bone is prepared according to a purification scheme similar to that described in Example I. The purification basically varies from that previously described by omission of the DE-52 column, the CM cellulose column and the mono s column, as well as a reversal in the order of the hydroxylapatite and heparin sepharose columns. Briefly, the concentrated crude 4 M extract is brought to 85% final concentration of ethanol at 4 degrees. The mixture is then centrifuged, and the precipitate redissolved in 50 mM Tris, 0.15 M NaCl, 6.0 M urea. This material is then fractionated on Heparin Sepharose as described. The Heparin bound material is fractionated on hydroxyapatite as described. The active fractions are pooled, concentrated, and fractionated on a high resolution gel filtration (TSK 30000 in 6 M guanidinium chloride, 50 mM Tris, pH 7.2). The active fractions are pooled, dialyzed against 0.1% TFA, and then fractionated on a C4 Vydac reverse phase column as described. The preparation is reduced and electrophoresed on an acrylamide gel. The protein corresponding to the 18K band is eluted and digested with trypsin. Tryptic fragments are isolated having the following amino acid sequences:

Fragment 9: S L K P S N H A T I Q S ? V

Fragment 10: S F D A Y Y C S ? A

Fragment 11: V Y P N M T V E S C A

Fragment 12: V D F A D I ? W

Tryptic Fragments 7 and 8 are noted to be substantially the same as Fragments 10 and 9, respectively.

A. bBMF-2

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Two probes consisting of pools of oligonucleotides are designed on the basis of the amino acid sequence of Fragment 3 and synthesized on an automated DNA synthesizer as described above.

Probe #1: A C N A C C A T [A/G] T C [T/C] T G [A/G] A T Probe #2: C A [A/G] G A [T/C] A T G G T N G T N G A

These probes are radioactively labeled and employed to screen the bovine genomic library constructed as follows: Bovine liver DNA is partially digested with the restriction endonuclease enzyme Sau 3A and sedimented through a sucrose gradient. Size fractionated DNA in the range of 15-30kb is then ligated to the lambda J1 BamH1 arms vector [Frischauf et al, J. Mol. Biol., 170:827-842 (1983) Mullins et al., Nature 308: 856-858 (1984)]. The library is plated at 8000 recombinants per plate. Duplicate nitrocellulose replicas of the plaques are made and amplified according to a modification of the procedure of Woo et al, <u>Proc. Natl. Acad. Sci. USA</u>, 75:3688-91 (1978).

The radioactively labelled 17-mer Probe #1 is hybridized to the set of filters according to the following method:

The probe is kinased and hybridized to the other set of filters in 3M tetramethylammonium chloride (TMAC), 0.1M sodium phosphate pH6.5, 1mM EDTA, 5X Denhardts, 0.6% SDS, 100ug/ml salmon sperm DNA at 48 degrees C, and washed in 3M TMAC, 50mM Tris pH8.0 at 50 degrees C. These conditions minimize the detection of mismatches to the probe pool [see, Wood et al, Proc. Natl. Acad. Sci, U.S.A., 82:1585-1588 (1985)]. 400,000 recombinants are screened by this procedure. One duplicate positive is plaque purified and the DNA is isolated from a plate lysate of the recombinant bacteriophage designated lambda bP-21. Bacteriophage bP-21 was deposited with the ATCC under accession number ATCC 40310 on March 6, 1987. The bP-21 clone encodes the bovine growth factor designated bBMP-2.

The oligonucleotide hybridizing region of this bBMP-2 clone is localized to an approximately 1.2 kb Sac I restriction fragment which is subcloned into M13 and sequenced by standard techniques. The partial DNA sequence and derived amino acid sequence of this Sac I fragment and the contiguous Hind III-Sac I restriction fragment of bP-21 are shown below in Table II. The bBMP-2 peptide sequence from this clone is 129 amino acids in length and is encoded by the DNA sequence from nucleotide #1 through nucleotide #387. The amino acid sequence corresponding to the tryptic fragment isolated from the bovine bone 28 to 30kd material is underlined in Table II. The underlined portion of the sequence corresponds to tryptic Fragment 3 above from which the oligonucleotide probes for bBMP-2 are designed. The predicted amino acid sequence indicates that tryptic Fragment 3 is preceded by a basic residue (K) as expected considering the specificity of trypsin. The arginine residue encoded by the CGT triplet is presumed to be the carboxy-terminus of the protein based on the presence of a stop codon (TAG) adjacent to it.

TABLE II

5	(1) GGC G	CAC H	GAT D	GGG G	15 AAA K	GGA	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	X X	45 CGG R
	CAA Q	GCA A	AAA K	CAC H	60 AAA K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
10	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	GTG V	GGG G	TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG P	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C	CAT H	180 GGG G
	GAG E		CCT P		195 CCC P	CTG L	GCC A	GAT D	CAC	210 CTT L	AAC N		ACG T	AAT N	225 CAT H
20		ATŤ I	V CTC	CATA Q	240 ACT T	CTG	GTC V	Aac N	TCA S	255 GTT V	AAC	TCT S	AAG K	ATT I	270 CCC P
25	AAG K	GCA A	TGC C	TGT C	385 GTC V	CCA	ACA T	GAG E	CTC L	300 AGC S	GCC A	ATC I	TCC S	ATG M	315 CTG L
			GAT D		330 AAT N	GAG	AAG K	GTG V	GTA V	345 TTA L	AAG K	AAC N	TAT Y	CAG	360 GAC D
30			GTC V			TGT	GGG G	TGT	ĊGT			397 GCA			07 TA
	TAA		417 ATA	TATA'		27 TA T	TAGA	43 AAAA		CAAA		TCA		457 GAC	
<i>35</i>	ACT		467 TAT	TTCC		77 GA A	ĢACT"	48 TTAT	7 T TA	TGGA	497 ATGG	TAA	GGAG	AAA	
	AAG		517 ACA	CAGC	5 TATT		AAAA			'ATA	547 TCTA			557 GAA	
40	GTT		567 AAA	CAAA		77 TT A	ATCA	58 GAGA		ATT					

EXAMPLE V

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Human Bone Inductive Factors

A. hBMP-2: Class I and II

The HindIII-SacI bovine genomic bBMP-2 fragment described in Example IV A. is subcloned into an M13 vector. A ³²p-labeled single-stranded DNA probe is made from a template preparation of this subclone. This probe is used to screen polyadenylated RNAs from various cell and tissue sources.

Polyadenylated RNAs from various cell and tissue sources are electrophoresed on formaldehyde-agarose gels and transferred to nitrocellulose by the method of Toole et al., supra. The probe is then hybridized to the nitrocellulose blot in 50% formamide, 5 X SSC, 0.1% SDS, 40 mM sodium phosphate pH6.5, 100 µg/ml denatured salmon sperm DNA, and 5 mM vanadyl ribonucleosides at 42° C overnight and washed at 65° C in 0.2 X SSC, 0.1% SDS. Following autoradiography, a hybridizing band corresponding to an mRNA species of approximately 3.8 kb is detected in the lane containing RNA from the human cell line U-2 OS. The HindIII-SacI fragment is labeled with 32P by nick translation and

used to screen the nitrocellulose filter replicas of a U-2 OS cDNA library by hybridization in standard hybridization buffer at 65° overnight followed by washing in 1 X SSC, 0.1% SDS at 65°.

This library was constructed by synthesizing cDNA from U-2 OS polyadenylated RNA and cloning into lambda gt10 by established techniques (Toole et al., supra). Twelve duplicate positive clones are picked and replated for secondaries. Duplicate nitrocellulose replicas are made of the secondary plates and both sets hybridized to the bovine genomic probe as the primary screening was performed. One set of filters is then washed in 1 X SSC, 0.1% SDS; the other in 0.1 X SSC, 0.1% SDS at 65°.

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Two classes of hBMP-2 cDNA clones are evident based on strong (4 recombinants) or weak (7 recombinants) hybridization signals under the more stringent washing conditions (0.1 X SSC, 0.1% SDS). All 11 recombinant bacteriophages are plaque purified, small scale DNA preparations made from plate lysates of each, and the inserts subcloned into pSP65 and into M13 for sequence analysis. Sequence analysis of the strongly hybridizing clones designated hBHP-2 Class I (also known as BMP-2) indicates that they have extensive sequence homology with the sequence given in Table II. These clones are therefore cDNA encoding the human equivalent of the protein encoded by the bBMP-2 gene whose partial sequence is given in Table II. Sequence analysis of the weakly hybridizing recombinants designated hBMP-2 Class II (also known as BMP-4) indicates that they are also quite homologous with the sequence given in Table II at the 3' end of their coding regions, but less so in the more 5' regions. Thus they encode a human protein of similar, though not identical, structure to that above.

Full length hBMP-2 Class I cDNA clones are obtained in the following manner. The 1.5 kb insert of one of the Class Il subclones (II-10-1) is isolated and radioactively labeled by nick-translation. One set of the nitrocellulose replicas of the U-2 OS cDNA library screened above (50 filters, corresponding to 1,000,000 recombinant bacteriophage) is rehybridized with this probe under stringent conditions (hybridization at 65° in standard hybridization buffer; washing at 65° in 0.2 X SSC, 0.1% SDS). All recombinants which hybridize to the bovine genomic probe which do not hybridize to the Class II probe are picked and plaque purified (10 recombinants). Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning into M13, sequence analysis indicates that 4 of these represent clones which overlap the original Class I clone. One of these, lambda U2OS-39, contains an approximately 1.5 kb insert and was deposited with the ATCC on June 16, 1987 under accession number 40345. The partial DNA sequence (compiled from lambda U2OS-39 and several other hBMP-2 Class I cDNA recombinants) and derived amino acid sequence are shown below in Table III. Lambda U2OS-39 is expected to contain all of the nucleotide sequence necessary to encode the entire human counterpart of the protein BMP-2 Class I encoded by the bovine gene segment whose partial sequence is presented in Table II. This human cDNA hBMP-2 Class I contains an open reading frame of 1188 bp, encoding a protein of 396 amino acids. This protein of 396 amino acids has a molecular weight of 45kd based on this amino acid sequence. It is contemplated that this sequence represents the primary translation product. The protein is preceded by a 5' untranslated region of 342 bp with stop codons in all frames. The 13 bp region preceding this 5' untranslated region represents a linker used in the cDNA cloning procedure.

TABLE III

5							
			0 30 T CAGCACITGG				70 AACTTGCGCA
				•			
10			0 100 C TTTGCCCAG			130 COGAGOCOCA	140 CCCCCTCC
•							
15		50 16 SC CTTGCCCGA	0 170 C ACTGAGACGC				210 GCCCGCACCC
	22						
	22 GGGAGAAGG		0 240 G AAAAGGAACG			270 GGICCITIGA	280 CCAGAGITIT
20	29	.0 30		220	220	240	252
			0 310 A ATGGACGIGT			GACTGCGGTC	350 TCCTAAAGGT
25	(1)		370	31	35	400	
	OGACC ATG	GTG GCC GG	ACC CGC TG Y Thr Arg Cy:	CIT CIA GO	E TIG CIG C	TT CCC CAG	GTC Val
							,
30	crc crc c	415 GC GGC GGG	SCT GGC CTC (130 FTT CCG GAG	CTG GGC CGC	AGG AAG TIV	c ccc
			Ala Gly Leu V	al Pro Glu	Leu Gly Arg	Arg Lys Phe	e Ala
	460 GCG GCG TI		175 DGC COOC TICA T	490	mom can can	505	2 63 6
35	Ala Ala S	er Ser Gly	irg Pro Ser S	Ser Gln Pro	Ser Asp Glu	Val Leu Sei	GAG Glu
		20	535		550		56 5
	TTC GAG T	re occ cre	TC AGC ATG	TC GGC CTG	AAA CAG AGA		C AGC
	File Giu II	eu Arg Leu .	eu Ser MET I	he Gly Leu	Lys Gln Arg	Pro Thr Pro	o Ser
40	100 010 0	580		595		610	
	Arg Asp A	cc GIG GIG (la Val Val I	CC CCC TAC A Pro Pro Tyr N	TG CIA GAC	CIG TAT CGC	AGG CAC TO	GGT
			10 110 171 1	mt ma wah	Ded Tyl Mig	ard ins ser	. GIÝ
46	625 CAG CCC C	ec mer cec	640 SCC CCA GAC (ne ee me	655	670)
45	Gln Pro G	ly Ser Pro	la Pro Asp F	lis Arg Leu	Glu Arg Ala	Ala Ser Arg	y Ala
		685		00	715		
	AAC ACT G	ng ogc age :	TC CAC CAT O The His His O	AA GAA TCT	TTG GAA GAA	CTA CCA GAZ	A ACC

	A	30 GT er	GGG	AAA Lys	ACA Thr	ACC Thr	745 C CGC Arc	AGA	TTC	TTC Phe	TIT	760 LAA 1 AST	TIA	AGI Ser	TCI Ser	'ATC	775	: ACC	GAC
5	G	AG lu	TTT Phe	790 ATC	ACC	TC?	GCA Ala	GAG	805 CIT	CAG	GTI Val	TTC Phe	C CGA	820 GAA Glu	CAG	AIG MEI	CAA	GAI Asp	835 GCI Ala
10	T L	ľA eu	GGA Gly	AAC Asn	AAT AST	850 AGC Ser	AGI	TIC Phe	CAT His	CAC His	865 CGA Arc	ITA Z	'AAT Asn	ATT Ile	TAT	880 GAA Glu	ATC	ATA Ile	AAA Lys
15	· O	CT ro	895 GCA Ala	ACA	GCC Ala	AAC Ast	TOS	910 AAA Lys	TTC	CCC Pro	: GIG Val	ACC Thr	925 AGI Ser	CIT Leu	TTG	GAC Asp	ACC Thr	940 AGG Arg	TIC
20	G V	rg al	AAT Asn	CAG Gln	955 AAT Asn	GCA	AGC Ser	AGG Arg	TCG	970 GAA Glu	AGI	TIII Phe	GAI Asp	GIC Val	985 ACC Thr	∞	GCI Ala	GIG Val	ATG MEI
20	α	g	TGG Trp	Thr	Ala	CAG Gln	Gly	CAC His	Ala	Asn	His	Glv	TTC	GIG Val	Val	GAA	1045 GIG Val	GCC	CAC His
25	T.	rG eu	GAG	1060 GAG Glu	AAA Lys	Gln	Gly	GIC	1075 TCC Ser	AAG Lys	AGA Arg	CAT His	GIT	1090 AGG Arg	ATA Ile	AGC Ser	AGG Arg	TCT	1105 TIG Leu
30	CZ H:	LS 	GIN.	GAT Asp	GAA	1120 CAC His	AGC Ser	Trp	TCA Ser	CAG	1135 ATA Ile	AGG Arg	Pro	TTG Leu	CTA	GTA Val	ACT	TTT Phe	GGC Gly
35	C? Hi	T	165 GAT Asp	GIY	Lys	GGG Gly	CAT	1180 CCT Pro	Leu	His	aaa Lys	AGA	1195 GAA Glu	AAA Lys	OGT Arg	CAA Gln	GCC	1210 AAA Lys	CAC
	тў	S	Gin	α	1225 AAA Lys	Arg	Leu	AAG Lys	TCC	L240 AGC Ser	TGT Cys	AAG Lys	AGA Arg	CAC His	CCT Pro	TTG Leu	TAC Tyr	GIG Val	GAC Asp
40	T	70 C e	AGI Ser	ASP	GTG Val	GGG	1285 TGG Trp	AAT Asn	GAC Asp	TGG Trp	ATT	I300 GTG Val	GCT Ala	ccc Pro	ccc Pro	GGG	I315 TAT Tyr	CAC His	GCC Ala
45	TT Ph	T ' e '	TAC	.330 TGC Cys	CAC His	GGA Gly	GAA Glu	TGC	345 CCT Pro	TTT Phe	CCT Pro	CTG Leu	GCT	L360 GAT Asp	CAT His	CIG Leu	AAC Asn	TCC	L375 ACT Thr
50	AA As	r (CAT His	GCC Ala	TTA	.390 GIT Val	CAG Gln	ACG Thr	TTG Leu	GIC	.405 AAC Asn	TCT Ser	GIT Val	AAC Asn	TCT	420 AAG Lys	ATT Ile	CCT Pro	AAG Lys

1435 1450 1465 1480
GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG
Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu

1495 1510 1525

AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGT TGT GGG
Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly

AAAA

Full-length hBMP-2Class II human cDNA clones are obtained in the following manner. The 200 bp EcoRI-Sacl fragment from the 5' end of the Class II recombinant II-10-1 is isolated from its plasmid subclone, labeled by nick-translation, and hybridized to a set of duplicate nitrocellulose replicas of the U-2 OS cDNA library (25 filters/set; representing 500,000 recombinants). Hybridization and washing are performed under stringent conditions as described above. 16 duplicate positives are picked and replated for secondaries. Nitrocellulose filter replicas of the secondary plates are made and hybridized to an oligonucleotide which was synthesized to correspond to the sequence of II-10-1 and is of the following sequence:

CGGGCGCTCAGGATACTCAAGACCAGTGCTG

Hybridization is in standard hybridization buffer at 50°C with washing at 50° in 1 X SSC, 0.1% SDS. 14 recombinant bacteriophages which hybridize to this oligonucleotide are plaque purified. Plate stocks are made and small scale bacteriophage DNA preparations made. After subcloning 3 of these into M13, sequence analysis indicates that they represent clones which overlap the original Class II clone. One of these, lambda U2OS-3, was deposited with the ATCC under accession number 40342 on June 16, 1987. U2OS-3 contains an insert of approximately 1.8 kb. The partial DNA sequence and derived amino acid sequence of U2OS-3 are shown below in Table IV. This clone is expected to contain all of the nucleotide sequence necessary to encode the entire human BMP-2 Class II protein. This cDNA contains an open reading frame of 1224 bp, encoding a protein of 408 amino acids, preceded by a 5' untranslated region of 394 bp with stop codons in all frames, and contains a 3' untranslated region of 308 bp following the in-frame stop codon. The 8 bp region preceding the 5' untranslated region represents a linker used in the cDNA cloning procedure. This protein of 408 amino acids has molecular weight of 47kd and is contemplated to represent the primary translation product.

TABLE IV

5	CTCTAGAG	10 3GG C	AGAG	20 Sagga		30 GGAGG		\GGAJ	40 GOGC		GCCC	50 GGC	CCGG		50 CA (GTGA	70
10	GCATCOGA	80 VGC I	CACC	90 SACGC		100 TGAGA		OGC.	110 IGCT			120 CIG	AGTA'		30 3C 1	rigici	140 100000
	l GATGGGAT	.50 TC C	CIC	160 CAAGC	TATCT	170 CGAGC		CAG	180 SC	ACA		190	GCCC:		00 XC 1	œiic	210 ACIG
15	2 CAACCGIT	20 CA G	AGGIY	230 CCCA	GGAGC	240 IGCIG		GCG/	250 4GCC	ŒC.		260 GCA	GGGA		70 NG (AGOCZ	280 TTCC
20	Z GIAGIGOO	90 AT C	XXXXX	300 CAAC		310 GCTGC		TTC	320 XIG	AGO	: CPPIN	330 CCA	GCAA		10 T T	CAAGA	350 YTTGG
25	3 CIGICAAG	60 AA T	CATGG	370 SACIG	TTATT	ÖBË ƏTATA			390 390	TGI		400 ACA	∞ À	l) IG AI ET II			,
<i>30</i> ·	417 GGT AAC Gly Asn	CGA . Arg :	ATG C	TG AT	432 TG GIC ET Val	GTT :	TTA Leu	TTA Leu	TGC Cys	447 CAA Gln	GTC Val	CIG Leu	CIA Leu	GGA Gly	462 GGC Gly	c ccc	,
	AGC CAT Ser His	GCT .	477 AGT I Ser I	TG A En I	TA CCT le Pro	GAG 2	492 ACG Ihr	GGG Gly	AAG Lys	AAA Lys	AAA Lys	507 GIC Val	GCC Ala	GAG Glu	ATI Ile	CAG Gln	
35	522 GGC CAC Gly His	GOG (Ala (GGA G Gly G	53 GA CC Sly Ar	c cc	TCA (Ser (GGG Gly	CAG Gln	552 AGC Ser	CAT His	GAG Glu	CIC Leu	CIG Leu	567 CGG Arg	GAC Asp	TTC Phe	
40	GAG GCG . Glu Ala	582 ACA Thr	CTT C	en Cl	G ATG n MET	597 TTT (Phe (GG Gly	CIG Leu	CGC Arg	CGC Arg	612 ŒC Arg	ccc Pro	CAG Gln	OCT Pro	AGC Ser	627 : AAG : Lys	
45	AGT GCC (GIC I	ATT C	42 CG GA TO As	C TAC p Tyr	ATG (œG (657 GAT Asp	CIT Leu	TAC Tyr	CGG Arg	CTT Leu	672 CAG Gln	TCT Ser	GGG Gly	GAG Glu	
50	687 GAG GAG (Glu Glu (GAA (Glu (GAG C Glu G	AG AI ln Il	702 C CAC e His	AGC A Ser I	ACT (Thr (GGT Gly	CIT	717 GAG Glu	TAT Tyr	CCT Pro	GAG Glu	œс	732	GCC	,

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				747					762					777					
	AGC	CGG	GΩ			GIG	AGG	AGC			CAC	CAA	GAA			CAC	ממ:	ATC	
	Ser	Arg	Ala	Asn	Thr	Val	Aru	Ser	Phe	His	His	Glu	Glu	His	Teu	Glu	Acn	Ile	
_		-																	
5	792					807	1				822					837			
	CCA	GGG	ACC	AGT	GAA	AAC	TCT	GCT	TIT	ŒĨ			TTT	AAC	CTC			ATC	
	Pro	Gly	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arq	Phe	Leu	Phe	Asn	Leu	Ser	Ser	Ile	
										_									
40			852					867					882					897	
10	CCI	GAG	AAC	GAG	GIG	ATC	TCC	TCT	GCA	GAG	CIT	ŒG	CTC	TIC	œ	GAG	CAG	GIG	
	Pro	Glu	Asn	Glu	Val	Ile	Ser	Ser	Ala	Glu	Leu	Arg	Leu	Phe	Arg	Glu	Gln	Val	
	63.6				912					927					942				
15	GAC	CAG	GGC	ccr	GAT'	TGG	GAA	AGG	GGC	TIC	CAC	ŒŢ	ATA	AAC	ATT	TAT	GAG	GIT	
15	Asp	GIN	Gly	Pro	Asp	Trp	Glu	Arg	Gly	Phe	His	Arg	Ile	Asn	Ile	Tyr	Glu	Val	
		057																	
	יצויה	957		~~3	CO3	~~~	972	~~~	~~~			987					1002		
	WETT	Tire	CCC Prom	Dm	NI-	CAA	GIG	GIG	CT.	GGG	CAC	CIC	AIC	ACA	CGA	CIA	CIG	GAC	
20		туз	PIO	PLO	ALG	GTIT	val	val	PIO	GIĀ	HIS	Ten	TTE	ınr	Arg	Leu	Leu	Asp	
				1017					1032					1047					
	ACG	AGA	CIG		CAC	·CAC	አልጥ			œc	TYCC.	באם.		1047	CAM	~	300	~~	
	In	Arq	Leu	Val	His	His	Asn	Val	Thr	Arrr	ינינים.	Glu	Thr	. Dha	gur	U2I	Sor	Dm	
			Leu	٠., -	.;;		131,711		\ \		. حوست		,11,14	,FIIC	بإثد		SĘI.	,FLO	Section .
25	106	2			-	1077					L092				•	1107			
	GCG	GIC	CIT	α c	TCC	ΑCC	œ	GAG	AAG	CAG	CCA	AAC	TAT	GGG	CTA	GCC	.ATT	GAG .	·
	Ala	Val	Leu	Arg	Trp	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu	;
													,	_					
	CTTC		1122					137]	1152				1	L167	
30	77-1	MCI.	LAL	Cit	CAT	CAG	ACT	ŒG	ACC	CAC	CAG	GGC	CAG	CAT	GIC	AGG	ATT	AGC	
-	. var	шп	His	LEU.	.rus	GIN	ınr	Arg	ınr	HIS	GLI	GLY	Gln	His	Val	Arg	Ile	Ser	
				7	182				-	197				,	222				
	CGA	TŒ	TTA			æ	ACT	ccc			circ	CAG	cur-	CCC 1	212	anc.	arc.	cmc	
25	Arq	Ser	Leu	Pro	Gln	Glv	Ser	Glv	Asn	LOG.	λla	CUD	Ten	Δm	Dm	Tau	LON	7721	
35	_					1		1				0111	LEG	ALG	FIO	Leu	Deu	vai	
	נ	227				נ	L242				נ	257				-	L272		
	ACC	TTT	GGC	CAT	CAT	GGC	α	GGC	CAT	GCC	TIG	ACC	CGA	œc	œ	AGG	GCC	AAG	
	Thr	Phe	Gly	His	Asp	Gly	Arg	Gly	His	Ala	Leu	Thr	Arq	Aru	Aru	Ara	Ala	Lvs	
40						_	_	-					- 3		5			-,,-	
40				287					.302				נ	317					
	œr	AGC	α	AAG	CAT	CAC	TCA	CAG	α	cc	AGG	AAG	AAG	AAT	AAG	AAC	TGC	α G	
	Arg	Ser	Pro	Lys	His	His	Ser	Gln	Arg	Ala	Arg	Lys	Lys	Asn	Lys	Asn	Cys	Arg	
	1222				_													•	
45	1332					347					362				נ	.377			
	3	CAC	TOG	CIC	TAT	GIG	GAC	TTC	AGC	GAT	GIG	GGC	TGG	TAA	GAC	TGG	TTA	GIG	
	Arg	nıs	Ser	ren	JYY	val	Asp	Pne	Ser	Asp	Val	Gly	Trp	Asn	Asp	Trp	Ile	Val	
		ר	1392				•	407				_	400				_	44-	
	CCC.			CCC	TTA C	CD C		407	m ~	m	ca m		422	~ ~~				.437	
50			CCA Pro																

	1452 GCT GAC CAC CTC AAC Ala Asp His Leu Asr	TCA ACC AAC Ser Thr Asn	CAT GCC ATT C	ETG CAG ACC CTG (FIC AAT TCT Val Asn Ser
5	1497 GTC AAT TCC AGT ATC Val Asn Ser Ser Ile	CCC AAA GCC	TGT TGT GTG C	527 CC ACT GAA CTG A	AGT GOC ATC
10	1557 TOC ATG CTG TAC CTG	l GAT GAG TAT	572 GAT AAG GTG G	1587 FIA CIG AAA AAT 1	PAT CAG GAG
15	ATG GTA GTA GAG GGA	1617 TGT GGG TGC	(408) 163 CCC TCACATCAC	36 1646	1656
	MET Val Val Glu Gly 1666 16 ATATACACAC CACACACA	76 1686	1696	1706 PACGITCCCA TCCACI	1716 1726 CACC CACACACTAC
20	1736 17 ACAGACIGCT TOCTTATA	46 1756 GC TGGACITITA	1766 TTTAAAAAA A	1776 NAAAAAAAAA AATGGA	1786 1796 AAAA ATCCCTAAAC
25	1806 18 ATTCACCITG ACCITATE	16 1826	1836	1846	1856 1866
30	1876 18 ATATATTTAT AACTAGT	86 1896 AT TAAAAGAAAA	1906 AAATAAAATG A	1916 GICATIATT TIAAAA	1926 1936 AAAA AAAAAAACT
	•				

1946 CTAGAGTOGA OGGAATTC

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The sequences of BMP-2 Class I and II as shown in Tables II, III IV and have significant homology to the beta (B) and beta (A) subunits of the inhibins. The inhibins are a family of hormones which are presently being investigated for use in contraception. See, A. J. Mason et al, Nature, 318:659-663 (1985). To a lesser extent they are also homologous to Mullerian inhibiting substance (MIS), a testicular glycoprotein that causes regression of the Mullerian duct during development of the male embryo and transforming growth factor-beta (TGF-b) which can inhibit or stimulate growth of cells or cause them to differentiate. Furthermore, the sequence of Table IV encoding hBMP-2 Class II has significant homology to the Drosophila decapentaplegic (DPP-C) locus transcript. See, J. Massague, Cell, 49:437-438 (1987); R. W. Padgett et al, Nature, 325:81-84 (1987); R.L. Cate et al, Cell 45: 685-698 (1986). It is considered possible therefore that BMP-2 Class II is the human homolog of the protein made from this transcript form this developmental mutant locus.

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EXAMPLE VI

Expression of Bone Inductive Factors.

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In order to produce bovine, human or other mammalian bone inductive factors, the DNA encoding it is transferred into an appropriate expression vector and introduced into mammalian cells by conventional genetic engineering techniques.

One skilled in the art can construct mammalian expression vectors by employing the sequence of Tables II, III AND IV or other modified sequences and known vectors, such as pCD [Okayama et al., Mol. Cell Biol., 2:161-170 (1982)] and pJL3, pJL4 [Gough et al., EMBO J., 4:645-653 (1985)]. The transformation of these vectors into appropriate host cells can result in expression of osteoinductive factors. One skilled in the art could manipulate the sequences of Tables II, III and IV by eliminating or replacing the mammalian regulatory sequences flanking the coding sequence with bacterial sequences to create bacterial vectors for intracellular or extracellular expression by bacterial cells. For ex-

ample, the coding sequences could be further manipulated (e.g. ligated to other known linkers or modified by deleting non-coding sequences there-from or altering nucleotides therein by other known techniques). The modified bone inductive factor coding sequence could then be inserted into a known bacterial vector using procedures such as described in T. Taniguchi et al., Proc. Natl Acad. Sci. USA, 77:5230-5233 (1980). This exemplary bacterial vector could then be transformed into bacterial host cells and bone inductive factor expressed thereby. For a strategy for producing extracellular expression of bone inductive factor in bacterial cells., see, e.g. European patent application EPA 177,343.

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Similar manipulations can be performed for the construction of an insect vector [see, e.g. procedures described in published European patent application 155,476] for expression in insect cells. A yeast vector could also be constructed employing yeast regulatory sequences for intracellular or extracellular expression of the factors of the present invention by yeast cells. [See, e.g., procedures described in published PCT application W086/00639 and European patent application EPA 123,289].

A method for producing high levels of an osteoinductive factor of the invention from mammalian cells involves the construction of cells containing multiple copies of the heterologous bone inductive factor gene. The heterologous gene can be linked to an amplifiable marker, e.g. the dihydrofolate reductase (DHFR) gene for which cells containing increased gene copies can be selected for propagation in increasing concentrations of methotrexate (MTX) according to the procedures of Kaufman and Sharp, <u>J. Mol. Biol.</u>, 159:601-629 (1982). This approach can be employed with a number of different cell types.

For example, a plasmid containing a DNA sequence for a bone inductive factor of the invention in operative association with other plasmid sequences enabling expression thereof and the DHFR expression plasmid pAdA26SV(A) 3 [Kaufman and Sharp, Mol. Cell. Biol., 2:1304 (1982)] can be co-introduced into DHFR-deficient CHO cells, DUKX-BII, by calcium phosphate coprecipitation and transfection. DHFR expressing transformants are selected for growth in alpha media with dialyzed fetal calf serum, and subsequently selected for amplification by growth in increasing concentrations of MTX (sequential steps in 0.02, 0.2, 1.0 and 5uM MTX) as described in Kaufman et al., Mol Cell Biol., 5: 1750 (1983). Transformants are cloned, and biologically active bone inductive factor expression is monitored by rat bone formation assay. Bone inductive factor expression should increase with increasing levels of MTX resistance. Similar procedures can be followed to produce other bone inductive factors.

Alternatively, the human gene is expressed directly, as described above. Active bone inductive factor may be produced in bacteria or yeast cells. However the presently preferred expression system for biologically active recombinant human bone inductive factor is stably transformed CHO cells.

As one specific example, to produce the human bone inductive factor (hBMP-1) of Example V, the insert of U2OS-1 is released from the vector arms by digestion with Sal I and subcloned into the mammalian expression vector pMT2CX digested with Xho I. Plasmid DNA from this subclone is transfected into COS cells by the DEAE-dextran procedure [Sompayrac and Danna PNAS 78:7575-7578 (1981); Luthman and Magnusson, Nucl. Acids Res. 11: 1295-1308 (1983)]. Serum-free 24 hr. conditioned medium is collected from the cells starting 40-70 hr. post-transfection.

The mammalian expression vector pMT2 Cla-Xho (pMT2 CX) is a derivative of p91023 (b) (Wong et al., Science 228:810-815, 1985) differing from the latter in that it contains the ampicillin resistance gene in place of the tetracycline resistance gene and further contains a Xhol site for insertion of cDNA clones. The functional elements of pMT2 Cla-Xho have been described (Kaufman, R.J., 1985, Proc. Natl. Acad. Sci. USA 82:689-693) and include the adenovirus VA genes, the SV40 origin of replication including the 72 bp enhancer, the adenovirus major late promoter including a 5' splice site and the majority of the adenovirus tripartite leader sequence present on adenovirus late mRNAs, a 3' splice acceptor site, a DHFR insert, the SV40 early polyadenylation site (SV40), and pBR322 sequences needed for propagation in E. coli.

Plasmid pMT2 Cla-Xho is obtained by EcoRl digestion of pMT2-VWF, which has been deposited with the American Type Culture Collection (ATCC), Rockville, MD (USA) under accession number ATCC 67122. EcoRl digestion excises the cDNA insert present in pMT2-VWF, yielding pMT2 in linear form which can be ligated and used to transform <u>E. coli</u> HB 101 or DH-5 to ampicillin resistance. Plasmid pMT2 DNA can be prepared by conventional methods. pMT2CX is then constructed by digesting pMT2 with Eco RV and Xbal, treating the digested DNA with Klenow fragment of DNA polymerase I, and ligating Cla linkers (NEBiolabs, CATCGATG). This removes bases 2266 to 2421 starting from the Hind III site near the SV40 origin of replication and enhancer sequences of pMT2. Plasmid DNA is then digested with EcoRl, blunted as above, and ligated to an EcoRl adapter,

5' PO4-AATTCCTCGAGAGCT 3'

3' GGAGCTCTCGA 5'

digested with Xhol, and ligated, yielding pMT2 Cla-Xho, which may then be used to transform <u>E. coli</u> to ampicillin resistance. Plasmid pMT2 Cla-Xho DNA may be prepared by conventional methods.

Example VII

Biological Activity of Expressed Bone Inductive Factor

5 A. BMP-1

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To measure the biological activity of the expressed bone inductive factor. (hBMP-1) obtained in Example VI above. The factor is partially purified on a Heparin Sepharose column. 4 ml of transfection supernatant from one 100 mm dish is concentrated approximately 10 fold by ultrafiltration on a YM 10 membrane and then dialyzed against 20mM Tris, 0.15 M NaCl, pH 7.4 (starting buffer). This material is then applied to a 1.1 ml Heparin Sepharose column in starting buffer. Unbound proteins are removed by an 8 ml wash of starting buffer, and bound proteins, including BMP-1, are desorbed by a 3-4 ml wash of 20 mM Tris, 2.0 M NaCl, pH 7.4.

The proteins bound by the Heparin column are concentrated approximately 10-fold on a Centricon 10 and the salt reduced by diafiltration with 0.1% trifluoroacetic acid. The appropriate amount of this solution is mixed with 20 mg of rat matrix and then assayed for <u>in vivo</u> bone and cartilage formation as previously described in Example III. A mock transfection supernatant fractionation is used as a control.

The implants containing rat matrix to which specific amounts of human BMP-1 have been added are removed from rats after seven days and processed for histological evaluation. Representative sections from each implant are stained for the presence of new bone mineral with von Kossa and acid fuschin, and for the presence of cartilage-specific matrix formation using toluidine blue. The types of cells present within the section, as well as the extent to which these cells display phenotype are evaluated.

Addition of human BMP-1 to the matrix material resulted in formation of cartilage-like nodules at 7 days post implantation. The chondroblast-type cells were recognizable by shape and expression of metachromatic matrix. The amount of activity observed for human BMP-1 was dependent upon the amount of human BMP-1 protein added to the matrix. Table IX illustrates the dose-response relationship of human BMP-1 protein to the amount of bone induction observed.

Table IX

	14510 17	
IMPLANT NUMBER	AMOUNT USED (equivalent of ml transfection media)	HISTOLOGICAL SCORE
876-134-1	10 BMP-1	C+2
876-134-2	3 BMP-1	C+1
876-134-3	1 BMP-1	C+/-
876-134-4	10 MOCK	C-
876-134-5	3 MOCK	C -
876-134-6	1 MOCK	· C-

Cartilage (c) activity was scored on a scale from 0(-) to 5.

Similar levels of activity are seen in the Heparin Sepharose fractionated COS cell extracts. Partial purification is accomplished in a similar manner as described above except that 6 M urea is included in all the buffers. Further, in a rat bone formation assay as described above, BMP-2 has similarly demonstrated chondrogenic activity.

The procedures described above may be employed to isolate other bone inductive factors of interest by utilizing the bovine bone inductive factors and/or human bone inductive factors as a probe source. Such other bone inductive factors may find similar utility in, inter alia, fracture repair.

The foregoing descriptions detail presently preferred embodiments of the present invention. Numerous modifications and variations in practice thereof are expected to occur to those skilled in the art upon consideration of these descriptions. Those modifications and variations are believed to be encompassed within the claims appended hereto.

50 Claims

Claims for the following Contracting States: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. A gene encoding human BMP-2 comprising the following DNA sequence:

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		1495 1510 1525 AAT GAA AAG GIT GTA TTA AAG AAC TAT CAG GAC ATG GIT GTG GAG GGT T Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly G	
10		1540(396) 1553 1563 1573 1583 1593 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AA Cys Arg	1603 YAAAAGAAA
15		AAAA	
	2.	2. A gene encoding human BMP-2 having the amino acid sequence given in claim 1.	
20	3.	3. A gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA seque	nce:
		 (a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of (b) which hybridises with a DNA sequence of claim 1 or section (a), above; or (c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said in above in the possible parameters. 	_
25		in changes in the peptide sequence or not.	
	4.	I. The DNA sequence of claim 3, which is a genomic DNA sequence.	
	5.	5. The DNA sequence of claim 3, which is a cDNA sequence.	
30	6.	6. A gene encoding bovine BMP-2 comprising the following DNA sequence:	
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	Ğ	L)	CAC H	GAT D	GGG G	15 AAA K		CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	AAG K	45 CGG R
5				AAA K	CAC H	60 AAA K				CGC R		AAG K	TCC S	AGC S	TGT C	90 AA G K
10	A(A	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	GTG V	GGG G	TGG W	AAT N	135 GAC D
15	TO W		ATC I		GCA A	150 CCG P	CCG P	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C	CAT H	180 GGG G
20	G <i>I</i> E	.G	TGC C	CCT P	TTT F			GCC A		CAC	210 CTT L	AAC N	TCC S	ACG T	AAT N	225 CAT H
	G(ATT I	CTC V	CAA Q				AAC N		255 GTT V		TCT S	`AAG K	ÁŤT I	270 CCC P
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25		•	GCA		TGT C				GAG			GCC	ATC I	TCC S	ATG M	315 CTG L
<i>25 30</i>	A <i>i</i> K	.G	GCA A	TGC C	TGT	GTC V	CCA P	ACA T	GAG E	CTC L	AGC S 345 TTA	GCC A	ATC I	S	M	CTG L
	A# K T# Y	.c	GCA A CTT L	TGC C GAT D	TGT C GAG	GTC V 330 AAT N 375	CCA P GAG E	ACA T AAG K	GAG E GTG V	CTC L GTA V	AGC S 345 TTA L	GCC A AAG K	ATC I AAC N	S TAT Y	M CAG O	CTG L 360 GAC D
30	AZ K TZ Y	.c	GCA A CTT L GTT V	TGC C GAT D GTC V	TGT C GAG E	GTC V 330 AAT N 375 GGT G	CCA P GAG E TGT C	ACA T AAG K GGG G	GAG E GTG V TGT C	CTC L GTA V (129 CGT R	AGC S 345 TTA L))	AAG K	ATC I AAC N 397 GCA A	S TAT Y	M CAG O 4(AAAA)	CTG L 360 GAC D
30	AJ K TJ Y	.G .C .C .AA	GCA A CTT L GTT V	GAT D GTC V 117 ATA 1	TGT C GAG E GAG E	GTC V 330 AAT N 375 GGT G	CCA P GAG E TGT C	ACA T AAG K GGG G	GAG E GTG V TGT C 437	CTC L GTA V (129 CGT R	AGC S 345 TTA L) TAGO	AAG K CACAC	ATC I AAC N B97 GCA I	TAT Y AAATA	CAG O AG AAAAT AAAAT	CTG L 360 GAC D
<i>30 35</i>	AI Y AT M	G G AA	GCA A CTT L GTT V TATA	GAT D GTC V 117 ATA 1	TGT C GAG E GAG E	GTC V 330 AAT N 375 GGT G AATAT	CCA P GAG E TGT C 7 A TT	ACA T AAG K GGG G	GAG E GTG V TGT C 437 AAAAC	CTC L GTA V (129 CGT R C AGO	AGC S 345 TTA L O) TAGC	AAG K CACAC 447 AAAA 497 ATGG	ATC I AAC N 397 GCA I	TAT Y AAATA	CAG Q 4(AAAA) 157 SAC 507 AAA	CTG L 360 GAC D

50 7. A gene encoding bovine BMP-2 containing the amino acid sequence of claim 6.

- 8. A gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:
 - (a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code;
 - (b) which hybridise with a DNA sequence of claim 7 or section (a), above; or
 - (c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not.

- 9. The DNA sequence of claim 8, which is a genomic DNA sequence.
- 10. The DNA sequence of claim 8, which is a cDNA sequence.

11. A gene encoding human BMP-4 comprising the following DNA sequence:

	10	20	30	40	50	. 60	70
	CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGOGC	GGAGCCCGGC	COGGAAGCTA	CCTCACTCTC
10							
	80	90	100	110	120	130	140
	GCATCOGAGC					AGTATCTAGC	TTGTCTCCCC
15	150	160	170	180	190	200	210
						COCTOCOC	
	220	230	240	250	260	270	280
20						GGGACCTATG	GAGCCATTCC
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5		Cly	ريد	AL 9	THE	LEU	PEI	vai	val	160	Leu	Cys	GII	vaı	Leu	Leu	GIA	GIĀ	Ala
					477					492					507				
		AGC	CAT	GCT	AGT	TIG	ATA	CI	GAG	ACG	GGG	AAG	AAA	AAA	GIC	GCC	GAG	TTA	CAG
		Ser	nis	Ald	ser	Leu	тте	Pro	GIU	'unr	GIA	Tys	Lys	Lys	Val	Ala	Glu	Ile	Gln
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		Gly	His	Ala	Gly	Gly	Arg	Arg	Ser	Gly	Gln	Ser	His	Glu	Leu	Leu	Arg	Asp	Phe
				582					597					612					627
		GAG	GCG	ACA	CIT	CIG	CAG	ATG	TTT	GGG	CIG	œc	œc	œc	∞	CAG	CT	AGC	AAG
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		Glu	Glu	Glu	Glu	Gln	Ile	His	Ser	Thr	GIV	Ten	GAG	TAI.	Pm	GAG	Am	Pm	GCC Ala
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25					747					762	!				777				
		AGC	œ	GCC	AAC	ACC	GIG	AGG	AGC	TTC	CAC	CAC	GAA	GAA	CAT	CTG	GAG	AAC	ATC
		Ser	Arg	Ala	Asn	Thr	Val	Arg	Ser	Phe	His	His	Glu	Glu	His	-Leu	-Glu	Asn	Ile
		792					807					822							
30				ACC	AGT	GAA		TCT	GCT	TTT	œı	TTC	CTC	יוידיני	AAC	CTC	837 AGC) ACC	ATC
		Pro	Gly	Thr	Ser	Glu	Asn	Ser	Ala	Phe	Arg	Phe	Leu	Phe	Asn	Leu	Ser	Ser	Ile
				852															
		CCT	GAG		GAG	GIG	ATC	TCC	867 TCT		GAG	سب	· ccc	882	uuv-	~~	CAC	CNC	897 GTG
35	•	Pro	Glu	Asn	Glu	Val	Ile	Ser	Ser	Ala	Glu	Leu	Arq	Leu	Phe	Arg	Glu	Gln	Val
													_						
		GAC	CAG	GGC	ССТТ	912 CAT	mee.	CAN) NGC	~~	927		~	3.003		942			GTT
		Asp	Gln	Gly	Pro	Asp	Trp	Glu	Arq	Gly	Phe	His	Ara	Ile	Asn	Tle	TAT.	Gli	Val
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		MET	Ivs	Pm	Pm	Ala	GAA	Val	GIG	Dm.	GGG	CAC	CIC	ATC	ACA	OGA A	CTA	CIG	GAC Asp
			1					· ·	٧۵١	110	GIY	шѕ	Leu	TIE	шк	Arg	Leu	Leu	Asp
45					1017					1032				_]	1047				
		ACG	AGA	CIG	GIC	CAC	CAC	AAT	GIG	ACA	œc	TGG	CAA	ACT	TTT	GAT	GIG	AGC	α
	٠٠	1111	Acg	Leu	vai	HIS	uis	ASN	vai	Thr	Arg	Trp	Glu	Túr.	Phe	Aşp	Val	Ser	Pro
		1062	2			נ	.077				:	1092				1	107		
50		GCC	CIC	CTT	œς	TCC	ÃΩ	∞	GAG	AAG	CAG	CCA	AAC	TAT	GGG	CTA.	GCC	ATT.	GAG.
30	•	Ala	Val	Leu	Arg	Trp	Thr	Arg	Glu	Lys	Gln	Pro	Asn	Tyr	Gly	Leu	Ala	Ile	Glu
			ו	122				7	.137				,	152					1
		GTG	ACT	CAC	CTC	CAT	CAG	ACT	CCG	ACC	CAC	CAG	GGC	.152 CAG	САТ	GIC	AGG	יוויוע.	.167 AGC
		Val	Thr	His	Leu	His	Gln	Thr	Arg	Thr	His	Gln	Gly	Gln	His	Val	Aru	Tle	Ser

	1182 1197 1212
	OGA TOG TTA OCT CAA GGG AGT GGG AAT TGG GCC CAG CTC CGG CCC CTC CTC CTC
5	Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
	1227 1242 1257 1272
	ACC TIT GGC CAT GAT GGC CGG GGC CAT GCC TIG ACC CGA CGC CGG AGG GCC AAG
	Thr Phe Gly His Asp Gly Arg Gly His Ala Leu Thr Arg Arg Arg Arg Ala Lys
10	1207
	1287 1302 1317
	OGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
	Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Asn Lys Asn Cys Arg
	1332 1347 1362 1377
15	1302
	CGC CAC TCG CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG Arg His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
	and the set with the per with and the way with the har
	1392 1407 1422 1437
	GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
20	Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cys His Gly Asp Cys Pro Phe Pro Leu
	1452 1467 1482
	GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT
	Ala Asp His Leu Asn Ser Thr Asn His Ala Ile Val Gln Thr Leu Val Asn Ser
25	1497 1512 1527 1542
	GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC
	Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
	1557 1572 1587
30	TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
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	1602 1617 (408) 1636 1646 1656
	ATG GTA GTA GAG GGA TGT GGG TGC OSC TGAGATCAGG CAGTCCTTGA GGATAGACAG
35	MET Val Val Glu Gly Cys Gly Cys Arg
•	1666 1676 1686 1696 1706 1716 1726
	ATATACACAC CACACACACA CACCACATAC ACCACACACA
	$oldsymbol{\cdot}$
40	1776 1746 1756 2756 2776
	1736 1746 1756 1766 1776 1786 1796 ACAGACTGCT TCCTTATAGC TGGACTTTTA TTTAAAAAA AAAAAAAAA AATGGAAAAA ATCCTAAAC
.:	Commission that the contract of
	1806 1816 1826 1836 1846 1856 1866
45	ATTCACCITG ACCITATIVA TGACITTACG TGCAAATGIT TTGACCATAT TGATCATATA TTTTGACAAA
•	The state of the s
	1876 1886 1896 1906 1916 1926 1936
	ATATATTAT AACTAGTAT TAAAAGAAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAAACT
50	
	1946
	CTAGAGTOGA CGGAATTC

12. A gene encoding human BMP-4 having the amino acid sequence given in claim 11.

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13. A gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA sequence:

- (a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;
- (b) which hybridises with a DNA sequence of claim 11 or section (a), above; or
- (c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results in changes in the peptide sequence or not.
- 14. The DNA sequence of claim 13, which is a genomic DNA sequence.
- 15. The DNA sequence of claim 13, which is a cDNA sequence.

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- **16.** A vector containing the gene or DNA sequence of any one of claims 1 to 15 in operative association with an expression control sequence.
- 17. A cell transformed with a vector of claim 16.

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- 18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
- 19. The cell of claim 18 which is a CHO cell.
- 20. A protein exhibiting properties of BMP-2 which is encoded by a gene or DNA sequence of any one of claims 1 to 10.
 - 21. A protein exhibiting properties of BMP-2, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 1 to 10, and recovering said protein from said culture medium.

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- 22. A protein exhibiting properties of BMP-4 which is encoded by a gene or DNA sequence of any one of claims 11 to 15.
- 23. A protein exhibiting properties of BMP-4, which is obtainable by the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence of any one of claims 11 to 15, and recovering said protein from said culture medium.
- 24. A process for producing the protein of claims 21 or 23, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.
- 25. A pharmaceutical composition comprising the proteins of any one of claims 20 to 23, individually or in combination, and a pharmaceutically acceptable vehicle.
 - **26.** The pharmaceutical composition of claim 25, further comprising a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.

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- 27. The pharmaceutical composition of claim 26, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.
- 28. Use of a protein of any one of claims 20 to 23, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

Claims for the following Contracting State: AT

50 1. A process for the preparation of a gene encoding human BMP-2 comprising the following DNA sequence:

	10	20	30	40	50	60	70
		GAGTGTGTGT					
5							
3							
	80		100	110		130	140
	CCCCACTITG	cccccccccc	TTTCCCCAG	CCCACCCTCC	TICCCCATCI	COGAGOCCCA	∞
10	• • • •						
10	150		170	180	190	200	210
	ACICCIOSC	CTTGCCCCAC	ACIGAGACGC	TGTTCCCAGC	GIGAAAAGAG	AGACIGOGOG	GCCCGCACCC
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15		GGAGGCAAAG				cerrerums)	
15		02200222	MANAGERICA	GACALICOGI	WII TOWN	GGICCIIIGA	CABABITIT
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		ere ecc ecc					
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	200 220 61	GIY ALE AL	a Gry Den v	ar Pro Gru	red Gry Mrg	Mry Lys M.	ie vre
	460	47	5	490		505	
30		TOS COC CO			TCT GAC GAG		C GAG
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		-			•		
•	520		535		550		565
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		500					
	NCC C3C C	580		595	~~~ m\m ~~	610	~ ~~
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5	Ser	G1	у Гу	rs Th	x Th	r Arg	J Ar	g Pho	e Ph	e Ph	e As	n Le	u Se	r Se	r Il	e Pro) Thr	Glu
			79					809	5				820)				835
	GAG	TT	T AT	C YC	CIC	A GCZ	GA(GCT	CAY	G GT	r TR	င္ကေတာ့	A GA	A CA	G AT	G CAA	GAT	COM
	GIU	ı Pn	е 11	e In	r Sei	r Ala	Gli	1 Lev	ı Cli	ı Va	l Phe	e Ar	g Glu	ı Gli	n ME	r Gln	Asp	Ala
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	Pro	Ala	a Thi	r Ala	a Ast	Ser	Lys	Phe	Pro	Val	Thr	: Sei	Lev	Lei	1 Ask	Thr	Ara	Leu
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	ana	ממ	ቦ ሮኔ/	955 מממי		300	300		970) 				985	5			
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25	Arg	Tr	Th	Ala	Gln	Gly	His	Ala	Asn	His	Glv	Phe	Val	Val	Glu	Val	λla	His
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	Teu	CAL	GAC	AAA	CAA	GGT	GIC	TCC	AAG	AGA	CAT	GII	' AGG	ATA	AGC	AGG	TCT	TTG
	Leu	GIU	L GIL	r. TAS	GIN	Gly	Val	Ser	Lys	Arg	His	Val	Arg	Ile	Ser	Arg	Ser	Leu
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	CAC	CAA	GAI	' GAA			TGG	מיאד	CAG	1135 מממ	acc.	~~	mm	~	1150	ACT		
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	CAT	GAT	GGA	AAA	GGG	CAT	α	CTC	CAC	AÀA	AGA	GAA	AAA	CCT	CAA	CCC	222	CAC
	His	Asp	Gly	Lys	Gly	His	Pro	Leu	His	Lys	Arg	Glu	Lys	Arg	Gln	Ala	Lys	His
													_				-	
	222	CAG		1225	~~	~~~]	240					L255				
40	Ivs	Gln	Arry	Tire	y	CIT	AAG T.~	100	AGC	IGT	AAG	AGA	CAC	ccr	TIG	TAC	GIG	GAC
	-3-	 .	9	Lys	My	Leu	TÀZ	Ser	ser	Cys	TÀZ	Arg	His	Pro	Leu	Tyr	Val .	Asp
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	TTC :	AGT	GAC	GTG			AAT	GAC	TGG	ATT	GTG.	CCT	\sim	œ.	CCC	1315 TAT	C2C /	~~
45	Phe :	Ser	Asp	Val	Gly	Trp .	Asn	ASD	Trp	Ile	Val	Ala	Pro	Pro	Glv	Tyr:	unc d Hie	BCC Bla
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1435 1480 1450 1465 GCA TGC TGT GTC COG ACA GAA CTC AGT GCT ATC TOG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Ieu Tyr Leu Asp Glu 5 1495 1510 1525 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGT TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1563 1573 1583 1593 1603 TGT GGC TÄGTÄCAGCA AAATTÄÄÄTÄ CATÄÄATATA TATATATATA TATATITTAG AAAAAAGAAA

15 AAAA

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wherein said process comprises the following steps:

- a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment by hybridization,
- b) isolating positive clones, and
- c) isolating the DNA-inserts from said clones.
- 2. The process according to claim 1, wherein the gene encodes human BMP-2 having the amino acid sequence given in claim 1.
 - 3. A process for the preparation of a gene encoding a protein exhibiting properties of human BMP-2 and comprising a DNA sequence:
 - a) which differs from a DNA sequence of claim 1 in codon sequence due to the degeneracy of the genetic code; ---b)-which-hybridizes with-a-DNA sequence of claim 1 or section (a), above; or
 - c) represents a fragment, allelic or other variation of a DNA sequence of claim 1, whether said variation results in changes in the peptide sequence or not,
- 35 wherein said process comprises standard techniques of molecular biology.
 - 4. The process according to claim 3, wherein the DNA sequence is a genomic DNA sequence.
 - 5. The process according to claim 3, wherein the DNA sequence is a cDNA sequence.
 - 6. A process for the preparation of a gene encoding bovine BMP-2 comprising the following DNA sequence:

5	(1) GGC G	CAC H	GAT D	GGG G	15 AAA K	GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	AAG K	45 CGG R
	CAA Q	GCA A	AAA K	CAC H	60 AAA K	CAG Q		AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 AAG K
10	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	gtg V	GGG G	TGG W	AAT N	135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG P	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C	CAT H	180 GGG G
20	GAG E	TGC C	CCT P		195 CCC P	CTG L	GCC A	GAT D	CAC H	210 CTT L	AAC N	TCC	ACG T	AAT N	225 CAT H
25	GCC A	ATT I	CTC V	CAA Q	240 ACT T	CTG: L	GTC V	AAC N	TCA S	255 GTT V	AAC N	TCT S	AAG K	ATT I	270 CCC P
	AAG K	GCA A	TGC C		385 GTC V			GAG E	CTC L	300 AGC S	GCC A	ATC I	TCC	ATG M	315 CTG L
30	TAC Y	CTT L	GAT D	GAG E		GAG E			GTA V	345 TTA L	AAG K	AAC N	TAT Y	CAG Q	360 GAC D
35	ATG M	GTT V	GTC			TGT		TGT	(129 CGT R	TAGO	CACAC	97 CA A	LAATA	40 LAAAL)7 'A
40	ТААА		17 TA T	'ATAT	42 'ATAT		'AGAA	437 AAAC			447 AAA	TCAA		57 AC	
•	ACTI				47 AATG	•			TAT		497 .TGG			07 AA	
45	AAGA			AGCT		7 T GA			TTT		547 CTA			57 AA	
	GTTG		67 AA C	AAAT	57 ATTT	7 T AA		587 AGAA		TT,					

wherein said process comprises the following steps:

a) screening a gene library constructed from bovine liver DNA or cDNA with a labelled probe designed on the basis of the amino acid sequence of a fragment of bBMP-2,

b) isolating positive clones, and

c) isolating the DNA-inserts from said clones.

^{7.} The process according to claim 6, wherein the gene encodes bovine BMP-2 having the amino acid sequence of claim 6.

	8.	A process for the preparation of a gene encoding a protein exhibiting properties of bovine BMP-2 and comprising DNA sequences:
5		a) which differ from a DNA sequence of claim 7 in codon sequence due to the degeneracy of the genetic code; b) which hybridize with a DNA sequence of claim 7 or section a), above; or c) represent fragments, allelic or other variations of a DNA sequence of claim 7, whether said variations result in changes in the peptide sequence or not,
10		wherein said process comprises standard techniques of molecular biology.
	9.	The process according to claim 8, wherein the DNA sequence is a genomic DNA sequence.
	10.	The process according to claim 8, wherein the DNA sequence is a cDNA sequence.
15	11.	A process for the preparation of a gene encoding human BMP-4 comprising the following DNA sequence:
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	10 20 CTCTAGAGGG CAGAGGAGGA		30 GGGAGGGAGG			cocèvecta e0			
5	80 GCATTOCGAGO	90 TGAGGGAGGC							
	150	160					210		
10	GATGGGATTC	COCTOCAAGC	TATCTCGAGC	CIGCAGOGOC	ACAGTOCCCG	GOCCIOGOCC	AGGITCACIG		
15	220 CAACOGTICA	230 GAGGTOOOCA		250 CTGGGGAGCC		-			
	290 GEAGIGOCAT	300 CCCCACCAAC			330 AGCCTTTCCA				
20	360 CIGICAAGAA	370 TCATGGACTG			400 TGTCAAGACA				
25	417		432	•	447		462		
	GGT AAC C Gly Asn A	CA ATG CTG urg MET Leu	ATG GTC GTT MET Val Val	. Leu Leu C	rs Gln Val 1	Leu Leu Gly	GGC GCG Gly Ala		
30	AGC CAT C	477 SCT AGT TIG Ma-Ser-Leu-	ATA CCT GAC -Ile Pro Glu	492 ACG GGG AI Thr-Gly-Ly	G AAA AAA Q	507 FTC GCC GAG Val Ala Glu	ATT CAG Ile Gln		
35	522 GGC CAC G Gly His A	GCG GCA GCA	537 CGC CGC TCF Arg Arg Ser	55 A GGG CAG AG Gly Gln Se	C CAT GAG	567 CTC CTG CGG Leu Leu Arg	GAC TTC Asp Phe		
	2. 1. 202 242	582 ACA CIT CIG	597 CAG ATG TT	r Gese cirs ex	612 SC GGC GGC (OG CAG OCT	627 AGC AAG		
40		thr Leu Leu 642 GTC ATT CCG		657		672	•		
	Ser Ala V	al Ile Pro	Asp Tyr MEI	Arg Asp Le	eu Tyr Arg	Leu Gln Ser	Gly Glu 732		
45	GAG GAG	GAA GAG CAG Glu Glu Glin	ATC CAC AGO	ACT GGT CI Thr Gly Le	T GAG TAT	CCT GAG CGC Pro Glu Arg	occ coc Pro Ala		
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_	ser	Arg	ATS	ASN	mr	var	Arg	Ser	Me	HIS	HIZ	GIU	GIU	HIS	Leu.	-GIU	ASN	Ile
5	792					807					822					837		
	∞ A	GGG	ACC	AGT	GAA	AAC	TCT	GCT	TTT	ŒĨ	TTC	CTC	TTT	AAC	CTC	AGC	AGC	ATC
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	Thr	Arg	Leu	Val	His	His	Asn	.Val	Thr	Arg	طئل	Glu	Thr	Phe	Asp	Val	Ser	Pro
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30	Val																	AGC Ser
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					182					1197		•			1212			
35	Œ A	TOG	TTA	α	CAA	œ	AGT	GGG	TAA	TGG	∞	CAG	cic	œ	∞	CIC	CIG	GTC
	Arg	Ser	Leu	Pro	Gln	Gly	Ser	Gly	Asn	Trp	Ala	Gln	Leu	Arg	Pro	Leu	Leu	Val
	1	227				3	242				2	1257					1272	
	ACC	TTT	GGC	CAT	GAT	GGC	ŒG	GGC	CAT	GCC	TIG	λ CC	∞A	œc	∞ G	AGG		AAG
40	Thr	Phe	Gly	His	Asp	Gly	Arg	Gly	His	Ala	Leu	Thr	Arg	Arg	Arg	Arg	Ala	Lys
			,	L287				,	L302					1317				
	-CCT	AGC			САТ	CAC	TCA			GCC	AGG	AAG			AAG	AAC	TGC	CGG
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			•		-1-	•					. —	2			•			
			1392					L407					1422					1437
50	GCC	CCA	₩.	GGC	TAC	CAG		IIC	TAC	TGC	CAT	GGG	GAC	TGC	CCC	TTT	CCA	CIG
	ALA	PTO	PTO	GIA	TVY	GIN	ALA	me	IVE	CVS	nıs	GIA	ASO	CVS	PIO	me	FIO	Leu
					145	12				146	57				148			
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F.F.	וב	a Ac	n Hi	SIP	או ויי	m 5e	T 11	IT AS	រាស	SAL	וג ב.	e va	لت ب	וג וג		iu ve		سه د د

	1497 1512 1527 1542 GTC AAT TOO AGT ATC OOO AAA GOO TGT TGT GTG OOO ACT GAA CTG AGT GOO ATC Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
5	val Asi Set Set Ite Pro Lys Ala Cys Cys val Pro Int Gid Led Set 124 225
-	1557 1572 1587
	TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG
	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
10	1602 1617 (408) 1636 1646 1656
	ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
	MET Val Val Glu Gly Cys Gly Cys Arg
	1666 1676 1686 1696 1706 1716 1726
15	7000 7070 7000
15	ATATACACAC CACACACACA CACCACATAC ACCACACACA
	1736 1746 1756 1766 1776 1786 1796
	ACAGACIGCI TOCTTATAGC TGGACTITTA TITAAAAAA AAAAAAAAA AATGGAAAAA ATOOCTAAAC
20	
	1806 1816 1826 1836 1846 1856 1866
	ATTCACCTIC ACCITATITA TGACITIACO TGCAAATGIT TIGACCATAT TGATCATATA TTITGACAAA
25	1876 1886 1896 1906 1916 1926 1936
	10/0 1000 1000 1000
	ATATATTTAT AACIAGIAT TAAAAGAAA AAATAAAATG AGTCATTATT TTAAAAAAA AAAAAAAACT
30	1946
	CIAGAGIOGA OGGAATIC,
	CIMINICA COMMIC,
	wherein said process comprises the following steps:
<i>35</i> ·	a) screening of a gene library constructed from U-2 OS derived DNA or cDNA with a labelled bBMP-2 fragment
	by hybridization,
	b) isolating positive clones, and
	c) isolating the DNA-inserts from said clones.
40	12. The process according to claim 11, wherein the gene encodes human BMP-4 having the amino acid sequence
70	given in claim 11.
	g. · · · · · · · · · · · · · · · · · · ·
	13. A process for the preparation of a gene encoding a protein exhibiting properties of BMP-4 and comprising a DNA
	sequence:
45	
	a) which differs from a DNA sequence of claim 11 in codon sequence due to the degeneracy of the genetic code;

wherein said process comprises standard techniques of molecular biology.

in changes in the peptide sequence or not,

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14. The process according to claim 13, wherein the DNA sequence is a genomic DNA sequence.

b) which hybridizes with DNA sequence of claim 11 or section a), above; or

- 15. The process according to claim 13, wherein the DNA sequence is a cDNA sequence.
 - **16.** A vector containing the gene or DNA sequence prepared according to any one of claims 1 to 15 in operative association with an expression control sequence.

c) represents a fragment, allelic or other variation of a DNA sequence of claim 11, whether said variation results

- 17. A cell transformed with a vector of claim 16.
- 18. The cell of claim 17 which is a mammalian cell, a bacterial cell, an insect cell or a yeast cell.
- 5 19. The cell of claim 18 which is a CHO cell.
 - 20. A process for the preparation of a protein exhibiting properties of BMP-2, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 1 to 10, and recovering said protein from said culture medium.
 - 21. A process for the preparation of a protein exhibiting properties of BMP-4, wherein said process comprises the steps of culturing in a suitable culture medium a cell transformed with an expression vector comprising a gene or a DNA sequence prepared according to any one of claims 11 to 15, and recovering said protein from said culture medium.
 - 22. A process for producing a protein exhibiting properties of BMP-2 or BMP-4, comprising the steps of culturing in a suitable culture medium the cell of claim 17 and isolating said protein from said culture medium.
- 20 23. A process for the preparation of a pharmaceutical composition comprising combining the proteins prepared according to any one of claims 20 to 22, individually or in combination with a pharmaceutically acceptable vehicle.
 - 24. The process according to claim 23, wherein said pharmaceutical composition further comprises a matrix capable of delivering the composition to the site of the bone or cartilage defect and providing a structure for inducing bone or cartilage formation.
 - 25. The process according to claim 24, wherein said matrix comprises hydroxyapatite, collagen, polylactic acid or tricalcium phosphate.
- 26. Use of a protein prepared according to any one of claims 20 to 22, individually or in combination, for the preparation of a pharmaceutical composition for inducing bone or cartilage formation.

Patentansprüche

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Patentansprüche für folgende Vertragsstaaten: BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Menschliches BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

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		1	LO		2	0			30			40			50)	
	GTCGAC	CTCTA	A GA	GTG	rgtg	T C	AGC	ACTI	'GG	CTG	GGA	CTT	CTI	GAA	CTT	3	
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	CCCNC	110			12		1001		.30	0000	2000	140		100m	150		
10	CGGAG	CTGC	J 1"1	CGC	CATC	T C	CGA	3CCC	CA	CCGC		TCC	ACI	CCT	CGGG	J	
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15	GCCGG	CACC	C GG	GAG	AAGO	SA G	GAG	GCAA	AG	AAA	AGGA	ACG	GAC	CATT	CGG!	r	
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5	GIL	PTO	GIA.	Ser	Pro	Ala	Pro	Asp	His	Arg	Leu	Glu	Arg	Ala	Ala	Ser	Arg	Ala
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	730					745					760					775		
	AGT	GGG	AAA	ACA	ACC	α G	AGA	TTC	TIC	TTT	AAT	TTA	AGT	TCT	ATC	∞	ACG	GAG
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	Leu	Gly	ASD	Asn	Ser	Ser	Phe	His	His	Arg	Ile	Asn	Ile	Tyr	Glu	Ile	He	Lys
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				955					970					985				
	GIG	TAA	CAG	TAA	GCA	AGC	AGG	TGG	GAA	AGI	The	GAT	GIC	ACC	D D D	Bla	172 J	ATG MET
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	Arg	Trp	Thr	Ala	Gln	Gly	His	s Ala	AST	His	Gly	Phe	: Val	Val	Glu	Val	Ala	His
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5		Phe	Tyr	CÀ2	His	Gly	Glu	CÀ2	Pro	Phe	ಮಾ	Leu	Ala	نحز	His	Leu	Asn	Ser	Thr	
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15					1495					1510					1525					
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		ASI	GTU	TÅR	var	val	Leu	TÀZ	AST	JÄL	GIN	ASP	WEIT.	vaT	Val	GIU	GIĀ	Cys	CIA	
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20					PACA	GCA 2	TAAA	TAAA'	IA C	ATAA	TAT	A TA	TATA'	CATA	TAT	ATTT	DAG .	AAAA	AAGAAA	•
		Cys	Arg																	
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25																				
	2.	Gen	, das r	nensc	hliche	s BMF	9-2 co	diert, c	das die	in Ar	spruc	h 1 an	geget	ene A	minos	säures	eque	nz aufv	weist.	
	2	Gen	dae d	in Pro	tein co	odiert	dae F	inense	hafte	n von i	manec	hliche	m RM	D_0 76	viat ur	nd aine	ם ראם	-80011	enz umfa	_

- Gen, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, und eine DNA-Sequenz umfaßt,
 die:
 - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet;
 - (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder
 - (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
 - 4. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
- 5. DNA-Sequenz nach Anspruch 3, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
 - 6. Rinder-BMP-2 codierendes Gen, umfassend die nachfolgende DNA-Sequenz:

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      (1)
      GGC CAC GAT GGG AAA GGA CAC CCT CTC CAC AGA AGA GAA AAG CGG
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          Α
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                      105
      AGA CAC CCT TTA TAT GTG GAC TTC AGT GAT GTG GGG TGG AAT GAC
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                  L
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      TGG ATC GTT GCA CCG CCG GGG TAT CAT GCC TTT
                                                                 G
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                                            Α
                      P
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                               G
                                   Y
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                                           210
                      195
      GAG TGC CCT TTT CCC CTG GCC GAT CAC CTT AAC TCC ACG AAT CAT
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                                                        Т
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      GCC ATT CTC CAA ACT CTG GTC AAC TCA GTT AAC TCT AAG ATT
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          CTT GAT GAG AAT GAG AAG GTG GTA TTA AAG AAC TAT CAG GAC
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             467
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     ACTITAMENT TECCCANTGN AGACTITATE ENEGGANTGG ANTGGAGANA
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             517
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     AAGAAAACA CAGCTATTTT GAAAACTATA TTTATATCTA CCGAAAAGAA
                                     587
                         577
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      GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,
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- 7. Gen, das Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 enthält.
- 55 8. Gen, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:
 - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 7 unterscheiden;

		(U) mill eme	I DIVA-S	edneuz i	iacii Alispi	ucii / odei i	acii voi	Stellenden	HUSAIZ	(a) Hybridisi	eren, oder	
5							ariationen ei ungen in der					rstellen, una	abhän-
	9.	DNA-9	Sequenz r	nach Ans	spruch 8,	dadurch g	ekennzeichn	et, daß	sie eine ge	enomisch	e DNA-Seq	uenz ist.	
	10.	. DNA-S	Sequenz r	nach Ans	spruch 8,	dadurch g	ekennzeichr	et, daß	sie eine cl	DNA-Seq	uenz ist.		
10	11.	. Menso	chliches B	MP-4 cc	dierende	s Gen, um	fassend die	nachfol	gende DNA	N-Sequen	z:		
		(CTCTAG	10 SAGGG	CAGA	20 GGAGGA	GGGAGG	30 GAGG	GAAGG	40 AGCGC	GGAGCC	50 CGGC	
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5		CGC	CGC	110 TGC		TCC			GTAT	_		TTGT		140 CCC		GGG	150 ATTC	
10		cco	TCC	160 AAG 0		TCT	17 CGAG		TGC		.80 CC			190 CCG	GCC	CTC	200 GCC0	
		AGO	STTC	210 ACTO		ACC	22 GTTC		AGG:		30 CA	GGAG		240 CTG		GCG	25 AGCC	50 2
15		CGC	TAC	260 TGC		GAC	_		AGC	_		GTAC					300 CAAC	-
		GC#	CTG	310 CTG(CTT			GCC.		30 CA			340 TGT		AGA'	350 TTG 0	
20		CTC	STCA	360 AGA		ATG			TAT:		80 TG			390 TTC		'CAA	400 GAC	-
25		cc		ATT								-						
	com	417															462	
30	Gly	ASD	Arg	MET	Leu	ATG MET	Val	GTT Val	TTA Leu	TTA Leu	TGC Cys	CAA Gln	Val	CTG Leu	CIA Leu	GGA Gly	GC	GCG Ala
	AGC Ser	CAT His	GCT Ala	477 AGT Ser	TIG Leu	ATA Ile	CCT Pro	GAG Glu	492 ACG Thr	GGG Gly	AAG Lys	aaa Lys	aaa Lys	507 GTC Val	GCC Ala	GAG Glu	ATT Ile	CAG Gln
35	522 GGC	CAC	ထေ	GCA	GGA.	537 CGC	œc	TCA	GGG	CAG	552 AGC	CAT	GAG	crc	cre	567 CGG	GAC	TTC
40	•		582					597					612				ASP	627
	Glu	Ala	Thr	Ten	Leu	Gln	MET	Phe	Gly	Leu	Arg	Arg	Arg	Pro	Gln	Pro	Ser	Lys
45	AGT Ser	GCC Ala	GTC Val	ATT Ile	642	GAC Asp	TAC Tyr	ATG MET	CCG Arg	657 GAT Asp	CIT Leu	TAC Tyr	CGG Arg	CTT Leu	672 CAG Gln	TCT Ser	GGG Gly	GAG Glu
	GAG Glu	687 GAG Glu	GAA Glu	GAG Glu	CAG Gln	ATC Ile	702 CAC His	AGC Ser	ACT Thir	GGT Gly	CIT Leu	717 GAG Glu	TAT Tyr	CCT Pro	GAG Glu	CGC Arg	732	GCC Ala
50				747					762	•				777				
	AGC Sei	Arg	Ala GCC	AAC ASD	ACC Thr	Val	AGG Arg	AGC Ser	TTC Phe	CAC His	CAC His	GAA Glu	GAA Glu	CAT His	CTG -Leu	GAG -Glu	AAC Asn	ATC

	792					807					822					837		
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5	Pro	Gly	Thr	Ser	Glu	ΆΣΩ	Ser	Ala	Pt.e	Arg	Phe	Leu	Phe	Asn	Leu	Ser	Ser	Ile
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15		957					972					987				7	1002	
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	Мa	Val	Leu	Arg	ŢŢ	Tri	Arg	Glu	Lys	Gln	520	Asn	Tyr	Gly	Leu	Ala	Ile	Glu
			1122					1137					1152				٠.	1167
	GTG			CTC	CAT	CAG			ACC	CAC	CAG			CAT	GIC	AGG		
30	Val	Thr	His	Leu	His	Gln	Thr	Arg	Thr	His	Gln	Gly	Gln	His	Val	Arg	Ile	Ser
50					1182					1197		_			212			
	CGA	TCG	TIA			œ	AGT	GGG	_		∞	CAG	CIC	ထင္ခေါ		CIC	CTG	GIC
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05		227														_		
35		227 ייייייי	GGC	СЪТ	CAT		1242	ccc	CAT	c~		1257	~~	~~	~~		272	AAG
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	ALY	SEL	PLO	LYS	MIS	HIS	Ser	GIN	Arg	ALA	Arg	Lys	TÀ2	Asn	TÀ2	ASN	Cys	Arg
	133	2				1347					1362					1377		
	ŒC	CAC	TŒ	CIC	TAT	GIG	GAC	TTC	AGC			GGC	TGG	AAT			ATT	GTG
45	Arg	His	Ser	Leu	Tyr	Val	Asp	Phe	Ser	Asp	Val	Gly	<b>Lib</b>	Asn	Asp	Trp	Ile	Val
			1392					3 40-					1 4 2 2					1427
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5	val Ash Ser Se	r tre to ras	HTM CAR CAR A	at Pro Lik Gr	I LEG SEL ALG THE
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	Ser MET Leu Ty	r Leu Asp Glu!	làr yab ràs M	al Val Len Ly:	s Asn Tyr Gln Glu
10			•		
	1602	1617	(408)		546 1656
		g GCA TGT GGG '		TCAGG CAGTCCT	nga ggatagacag
	MET Val Val Gl	u Gly Cys Gly	Cys Arg		
15					
	1666	1676	1686	1696	1706
		CACACACACA C			<del>-</del> · · · -
	ATATACACAC	CACACACACA	ACCACATAC A	HCCHCHCHCH (	CACGITCCCA
20		1776	1726	1746	1756
	1710	5 1726 C CACACACTAC	1736		—
	TCCACTCACC	CACACACTAC	ACAGACIGCI	ICCITATAGE	IGGACIIIIA
		1226	1706	1796	1806
	1766	1776	1786		
25	'I"I"I'AAAAAAA	AAAAAAAAA	AATGGAAAAA	ATCCCTAAAC A	RITCACCITG
23			1026	1046	1056
	1816	1826	1836	1846	1856
	ACCTTATTTA	TGACTTTACG T	I'GCAAATGTT	TIGACCATAT	IGATCATATA
			1006	1006	1006
20	1866	1876	1886	1896	1906
30	TTTTGACAAA	ATATATTTAT A	AACTACGTAT '	TAAAAGAAAA A	AAATAAAATG
	1916	1926	1936	1946	20011550
	AGTCATTATT	TTAAAAAAAA A	AAAAAAAAC'I'	CTAGAGTCGA (	CGGAATTC
05					
35	40.0	5145 4 # 1			
	12. Gen, das menschliche	s BMP-4 codient, das	die in Anspruch 11	angegebene Amino	sauresequenz aufweist.
	13. Gen, das ein Protein o	odiert, das Eigenscha	aften von BMP-4 ze	eigt, und eine DNA-S	equenz umfaßt, die:
40			ler Degeneriertheit	des genetischen Co	odes von einer DNA-sequenz
	nach Anspruch 11	unterscheidet;			*
	(b) mit einer DNA-	Sequenz nach Anspr	uch 11 oder nach v	orstehendem Absatz	z (a) hybridisiert; oder
45	(c) ein Fragment	aina allalischa odar ai	ina andora Variatios	a ainar DNA-Saguar	z nach Anenruch 11 daretallt

- (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht.
  - 14. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine genomische DNA-Sequenz ist.
- 50 15. DNA-Sequenz nach Anspruch 13, dadurch gekennzeichnet, daß sie eine cDNA-Sequenz ist.
  - **16.** Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.
- 17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.
  - 18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.

- 19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.
- Protein, das Eigenschaften von BMP-2 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 codiert ist.
- 21. Protein, das Eigenschaften von BMP-2 aufweist, das erhältlich ist durch die Schritte
  - Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 1 bis 10 umfaßt, und
  - Gewinnen des Proteins aus dem Kulturmedium.
- Protein, das Eigenschaften von BMP-4 aufweist, das durch ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 codiert ist.
- 23. Protein, das Eigenschaften von BMP-4 aufweist, das erhältlich ist durch die Schritte
  - Züchten einer mit einem Expresionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Vektor ein Gen oder eine DNA-Sequenz nach einem der Ansprüche 11 bis 15 umfaßt und
  - Isolieren des Proteins aus dem Kulturmedium.
- 24. Verfahren zur Herstellung des Proteins nach Anspruch 21 oder 23, umfassend die Schritte
- Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
  - Gewinnen des Proteins aus dem Kulturmedium.
- 25. Arzneimittel, dadurch gekennzeichnet, daß es, einzeln oder in Kombination, die Proteine nach einem der Ansprüche 20 bis 23 und einen pharmakologisch verträglichen Träger umfaßt.
  - 26. Arzneimittel nach Anspruch 25, dadurch gekennzeichnet, daß es ferner eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.
  - 27. Arzneimittel nach Anspruch 26, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.
- **28.** Verwendung des Proteins nach einem der Ansprüche 20 bis 23, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

### Patentansprüche für folgenden Vertragsstaat : AT

 Verfahren zur Herstellung eines menschliches BMP-2 codierenden Gens, das die nachfolgende DNA-Sequenz umfaßt:

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	1	0 20	30	40	50	
	GTCGACTCTA	GAGTGTGTGT	CAGCACTTGG	CTGGGGACTT	CTTGAACTTG	
5	6		80		100	
	CAGGGAGAAT	AACTTGCGCA	CCCCACTTTG	CGCCGGTGCC	TTTGCCCCAG	
	110				150	
	CGGAGCCTGC	TTCGCCATCT	CCGAGCCCCA	CCGCCCCTCC	ACTCCTCGGC	
10	160	170	180	190	200	
	CTTGCCCGAC	ACTGAGACGC	TGTTCCCAGC	GTGAAAAGAG	AGACTGCGCG	
	210	220	230	240	250	
15	GCCGGCACCC	GGGAGAAGGA	GGAGGCAAAG	AAAAGGAACG	GACATTCGGT	
	260				300	
	CCTTGCGCCA	GGTCCTTTGA	CCAGAGTTTT	TCCATGTGGA	CGCTCTTTCA	•
20	310					
	ATGGACGTGT	CCCCGCGTGC	TTCTTAGACG	GACTGCGGTC	TCCTAAAGGT	
•	(1)	370		385	40	O
25	CGACC ATG GT	G GCC GGG ACC	व्हट गढां टाग	CIA GOS TIG CI	RCIT CCC CA	GTC
20	LIET AG	r wra gry inc	Arg Cys Leu	Leu Ala Leu Le	en l'en Pro Gli	n Val
		415	430		445	
	CIC CIG GGC	eec ece ect e	SC CIC GIT CO	G GAG CITG GGC	CCC ACC AAC	TTC GOG
30	red red Gry	GIA WIG WIG C	TA TEST AST DA	o Glu Leu Gly	Arg Arg Lys	Pne Ala
	460	475		490	505	
	ece ece tee	$ar{x}$ eec eec o	CC TCA TCC CA	G CCC TCT GAC	GAG GTC CTG	age gag
	Ala Ala Ser	Ser Gly Arg P	ro Ser Ser Gl	n Pro Ser Asp	Glu Val Leu	Ser Glu
35	520		535	550		565
	TIC GAG ITG	ace are are y	GC ATG TTC GG	ic ctg aaa cag	AGA CCC ACC	CCC AGO
	ene Glu Leu	Arg Leu Leu S	er MET Phe Gl	y Leu Lys Gln	Arg Pro Thr	Pro Ser
		580	59		610	
40	has say so	ere ere exe e	ac are ci	A GAC CTG TAT	OCC AGG CAC	TOG GGT
	arg Asp Ala	Val Val Pro P	ro Tyr MET Le	u Asp Leu Tyr	Ard Ard His	Ser Glv

		625					640					655					670	
	CAG	<u></u>	GGC	TCA	$\infty$	έœ	œχ	GAC	CAC	œ	TIG	GYC	AGG	GCA	GCC	AGC	<b>Œ</b> A	œ
5	GIN	PTO	GIÀ	Ser	Pro	ALa	Pro	yzb	His	Arg	Leu	Glu	Arg	Ala	Ala	Ser	Arg	Ala
				685					700					715				
	AAC	ACT	GIG		AGC	TTC	CAC	САТ		CAA	بلبك	ىكىك	CAA		CTA	CCA	GLA	ACG
	Asn	Thr	Val	Aru	Ser	Phe	His	Fis	Glu	Glu	Ser	Leu	Glu	Glu	Leu	Pro	Glu	Thr
								-								• • •		
10	730					745					760					775		
		GGG	AAA	<b>2</b> C2	ACC		<b>JCJ</b>	יאווני	TIC	ידיד		TTA	AGT	TCT	ATC		ACG	GAG
	Ser	Glv	Lys	Thr	Thr	Aru	Arcı	Phe	Phe	Phe	Asn	Leu	Ser	Ser	Ile	Pro	Thr	Glu
		•					,									_		
			790					805					820					835
15			ATC															
	Glu	Phe	Ile	Inr	Ser	Ala	Glu	Leu	GID	Val	Pne	Arg	GIU	GIN	MET	GIN	Asp	Ala
					850					865					880			
	TTA	GGA	AAC	AAT			TTC	CAT	CAC			AAT	ATT	TAT		ATC	ATA	AAA
20	Leu	Gly	Asn	Asn	Ser	Ser	Phe	His	His	Arg	Ile	ASD	Ile	Tyr	Glu	Ile	Ile	Lys
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	Pro	ALA	TUL	ALA	ASN	ser	TĀR	Pne	PIC	Val	ш	Ser	ושנו	וופענ	, ASp	1177	Mry	Leu
25				955					970	<b>)</b>				985				
	GTG	AAT	CAG			AGC	AGG	TGG			TTT	GAI	GIC			GCT	GIG	ATG
	Val	AST	Gln	AST	Ala	Ser	Arg	Tr	Glu	Ser	Phe	: Asp	Val	Thr	Pro	Ala	. Val	MET
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	100					1015					1030			. ~~		1045		· ma
30	OGG	TGG	ACI	GCA	CAG	GGA	CAC	: GO	AAC	CAI	GG	TIC	: GIG	1757	GAA	GIG	7.1	CAC
	Arg	irr	, 1111	. Ale	LGLI	r eră	HIE	ALE	AST	ınıs	, GT	, me	· · · ·	Val	GIU	ı val	. Ale	His
			1060				•	1075					1090					1105
•	TIG	GAC			CAA	. GG	GI			AG	A CAI	GI			AGC	: AGG	TCI	TIG
35	Leu	Glu	ı Glu	ı Lys	: Glr	Gly	Val	L Ser	: Ly:	s Arg	, His	· Val	Arc	, Ile	Ser	Arg	Ser	Leu
						_					_							
	C3.C	. ~ .	C 3.5	. ~ .	1120			- ~~		1139		- ~~		- ~	1150		• 1111	
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			ردم ،	, Gr	4 1112	3 SEI	- 111	با حود	. GL		- <b></b> .	3 220						
40		1165	5				1180	0				1195	5				1210	)
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	His	As	o Gly	/ Ly:	s Gly	/ His	s Pro	o Lea	i Hi	s Ly:	s Arg	g Glu	ı Ly:	s Arq	g Glr	n Ale	I Ly:	: His
				122	=				224	^				125				
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	12	70				128	5				130	0				131	5	
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50	Ph	e Se	r As	sv og	ı Gl	y Tr	p As	an As	P T	p Il	.e Va	וג נו	a Pr	rq or	ro Gl	у ту	r Ki	s Ala
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	===	TT	ic To	SC C	C CC	iA G	T Ai	sc a	ar I	er c	II (	le e	II G		بت ت	נא פנ	C I	C ACT
	-77	e T	L C	/s Hi	LS G]	Ly G	ıu C	ys P	m ?	ne Pi	ro L	eu Al	La A	50 H	15 14	کتر باک	5T. S	er Thr
					139	90				140	15				14:	20		
55	22	TC	T GO	n at			ig a	ag m	יום כי			ar G	er a	ac D			er o	CT AAG
																		ro Lys

1435 1450 1465 1480 GCA TGC TGT GTC COG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1525 1495 1510 aat gaa aag git gia tia aag aac tat cag gac atg git gig gag ggt igi ggg Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1583 1593 1603 1563 1573 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTITTAG AAAAAAGAAA Cys Arg 15 AAAA. wobei das Verfahren die nachfolgenden Schritte umfaßt: (a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank 20 aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war, (b) Isolieren positiver Clone und (c) Isolieren der DNA-Insertionen aus diesen Clonen. 25 2. Verfahren nach Anspruch 1, dadurch gekennzeichnet, daß das Gen menschliches BMP-2 codiert, das die in Anspruch 1 angegebene Aminosäuresequenz aufweist. Verfahren zur Herstellung eines Gens, das ein Protein codiert, das Eigenschaften von menschlichem BMP-2 zeigt, 30 und eine DNA-Sequenz umfaßt, die: (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 1 unterscheidet; 35 (b) mit einer DNA-Sequenz nach Anspruch 1 oder nach vorstehendem Absatz (a) hybridisiert; oder (c) ein Fragment, eine allelische oder eine andere Variation einer DNA-Sequenz nach Anspruch 1 darstellt, unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht, 40 wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist. Verfahren nach Anspruch 3, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist. 45 Verfahren zur Herstellung eines Rinder-BMP-2 codierenden Gens, umfassend die nachfolgende DNA-Sequenz: 50

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5	(1) GGC G	CAC H	GAT D			GGA G	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	AAG K	45 CGG R
				CAC H	60 AAA X	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG K	TCC S	AGC S	TGT C	90 <b>AAG</b> K
10	AGA R	-CAC H	-CCT P	TTA L	105 TAT Y			TTC F		120 GAT D	GTG V	-GGG G	TGG W	AAT N	135 GAC D
15		ATC I	GTT V	GCA A	150 CCG P	CCG P			CAT H	165 GCC A		TAC Y	TGC C	CAT H	180 GGG G
20	GAG E	TGC C	CCT P	TIT F	195 CCC P	CTG L	GCC A	GAT D	CAC H	210 CTT L		TCC	ACG T	AAT N	225 CAT H
25		[:] Att I	CTC V	CXA Q	T	CTG L	V	N	S	<b>V</b>	AAC N	S	``AAG K	Ξ	₽
	AAG K	GCA A		TGT C				GAG E					TCC S		
30	TAC Y	CTI L	GAT D	−GAG E	330 - AAT N		AAG X	GTG V	GTA V	345 TTA L		AAC <u>Y</u>	TAT Y	CAG O	360 GAC D
35	ATO M	GTT	GTC V	GAG E			G G			9) TAG		397 :GCA	AAAT		07 .TA
40	TA	AATA'	417 IATA	TAT	ATAT	427 ATA	TTAG.	4: AAAA	37 AC A		44 AAAA		CAAGT	457 TGAC	
45	AC	TTTA	TATA	TTC	CCAA'	TGA	AGAC	TTTA'	TT T	ATGG	AATO	ig aj	ATGG	KGAAA	
	AA	GAAA.	517 AACA					5 ACTA					CGAA	557 \AGA	
50	GT'	TGGG.		CAA			AATC	5 AGAG	87 AA T	TATI	•				_

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- (a) Absuchen einer Genbank mit einer markierten auf der Grundlage der Aminosäuresequenz eines Fragmentes von bBMP-2 entworfenen Sonde, wobei die Genbank aus Rinderleber-DNA oder cDNA konstruiert wurde,
  - (b) Isolieren positiver Clone und

- (c) Isolieren der DNA-Insertionen aus diesen Clonen.
- 7. Verfahren nach Anspruch 6, dadurch gekennzeichnet, daß das Gen Rinder-BMP-2 codiert, das die Aminosäuresequenz von Anspruch 6 aufweist.
- 8. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von Rinder-BMP-2 zeigt, und DNA-Sequenzen umfaßt, die:
- (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz 10 nach Anspruch 7 unterscheiden;
  - (b) mit einer DNA-Sequenz nach Anspruch 7 oder nach vorstehendem Absatz (a) hybridisieren; oder
  - (c) Fragmente, allelische oder andere Variationen einer DNA-Sequenz nach Anspruch 7 darstellen, unabhängig davon, ob die Variationen zu Änderungen in der Peptidsequenz führen oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

- 9. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.
- 10. Verfahren nach Anspruch 8, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.
- 11. Verfahren zur Herstellung eines menschliches BMP-4 codierenden Genes, das die nachfolgende DNA-Sequenz umfaßt:

25	10	20	30	40	50
	CTCTAGAGGG	CAGAGGAGGA	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCGGC
30	60	70	80	90	100
	CCGGAAGCTA	GGTGAGTGTG	GCATCCGAGC	TGAGGGACGC	GAGCCTGAGA
	110	120	130	140	150
	CGCCGCTGCT	GCTCCGGCTG	AGTATCTAGC	TTGTCTCCCC	GATGGGATTC
35	160	170	180	190	200
	CCGTCCAAGC	TATCTCGAGC	CTGCAGCGCC	ACAGTCCCCG	GCCCTCGCCC
	210 AGGTTCACTG	220 CAACCGTTCA	230 GAGGTCCCCA		250 CTGGCGAGCC
40	260	270	280	290	300
	CGCTACTGCA	GGGACCTATG	GAGCCATTCC	GTAGTGCCAT	CCCGAGCAAC
45	310	320	330	340	350
	GCACTGCTGC	AGCTTCCCTG	AGCCTTTCCA	GCAAGTTTGT	TCAAGATTGG
	360 CTCTCAAGAA	370 TCATGGACTO			400 TGTCAAGACA

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## (1) CC ATG ATT CCT MET Ile Pro

3																			
	GT Gly	417 AAC ASTI	yrd ŒY	AIG MET	CIG CIG	atg Yet	432 GIC Val	GIT Val	TIA Leu	TTA Leu	cře lec	447 CAA Gln	Asj GLC	CTG Leu	CIA Leu	GGA Gly	462 GGC Gly	ecc Ala	
10	AGC Ser	CAT His	GCT Ala	477 AGT Ser	TIG Leu	ATA Ile	CCT Pro	CAG Glu	492 ACG Thr	GGG Gly	lys Aac	AAA Lys	AAA Lys	507 GTC Val	c∝ Ala	Ç≟G Glu	ATT Ile	CAG Gln	
15	522 GGC Gly	CAC	GCG Ala	Gly GGA	GJÀ ∝y	537 CGC Arg	CGC Arg	TCA Ser	GS Gly	CAG Gln	552 AGC Ser	CAT His	GAG Glu	CTC Leu	CTG Leu	557 CCG Arg	پخت <i>وح</i> ر	TTC Phe	
20	GAG Glu	GCC Ala	582 ACA	CIT	CTG Leu	G]n CAG	aig Met	597 TIT Phe	es Gly	CIG Leu	CSC Arg	CGC Arg	612 CGC Arg	CCG Pro	CAG Gln	CCT Pro	AGC Ser	627 AAG Lys	
	AGT Ser	G∝ Ala	GTC Val	ATT Ile	642 CCG Pro	GYC Yeb	TAC Tyr	atg Met	yrd cc:	657 CAT Asp	CTT Leu	TAC Tyr	CGG Arg	CTT Leu	672 CAG Gln	TCI	GGG	CAG Glu	
25	GAG Glu	687 GAC	CAA	G}u	CAG Glin	ATC Ile	702 CAC His	AGC Ser	ACT This	GT Gly	CTT Leu	717 GAG Glu	TAT Tyr	CCT Pro	GAG Glu	್ರ ೧೦೦೦	732	€ Ala	
30					c acc					CAC					CIC			ATC	
35	79: ©	2 AGG	G AC	c ag:	r GYI	807 AAC	rci	r 601	r	യോ	822 TIC		TI	C AAC	c cro	837 AGC	7 C AGO	C ATC	
40				C GA					r GCZ					TI				897 G GTG n Val	
						rac					c ca		_		_	r TAI	_	GTT 1 Val	
45			$\infty$					GI					) AIK					1 y25 3 CYC 3	
50					د حر					y cc					r GA			= 3m c ccr	
55		G CI										a aa					C.AT	r GAG. e Glu	

	1122 1137 1152 1167
	GIE YOU CYC CUC CYL CYE YOU COE YOU CYC CYC GGC CYC CYL GIC YES YILL YEC
	Val The His Leu His Gln The Arg The His Gln Gly Gln His Val Arg Ile Ser
5	1182 1197 1212
	CCA TOS TTA COT CAA CCC AGT CCG AAT TGG CCC CAG CTC CCC CCC CTC CTC GTC
	Arg Ser Leu Pro Gln Gly Ser Gly Asn Trp Ala Gln Leu Arg Pro Leu Leu Val
	1227 1242 1257 1272
10	1227 1242 1257 1272 ACC TIT GGC CAT GAT GGC GGG GGC CAT GCC TIG ACC CGA CGC CGG AGG GCC AAG
	The Pine Gly His Asp Gly Arg Gly His Ala Leu The Arg Arg Arg Ala Lys
	1287 1302 1317
15	CGT AGC CCT AAG CAT CAC TCA CAG CGG GCC AGG AAG AAG AAT AAG AAC TGC CGG
	Arg Ser Pro Lys His His Ser Gln Arg Ala Arg Lys Lys Asn Lys Asn Cys Arg
•	1332 1347 1362 1377
	CCC CAC TOS CTC TAT GTG GAC TTC AGC GAT GTG GGC TGG AAT GAC TGG ATT GTG
20	Ary His Ser Leu Tyr Val Asp Phe Ser Asp Val Gly Trp Asn Asp Trp Ile Val
	7.422
	1392 1407 1422 1437 GCC CCA CCA GGC TAC CAG GCC TTC TAC TGC CAT GGG GAC TGC CCC TTT CCA CTG
	Ala Pro Pro Gly Tyr Gln Ala Phe Tyr Cvs His Gly Aso Cvs Pro Phe Pro Leu
25	3.402
	1452 1467 1482
	GCT GAC CAC CTC AAC TCA ACC AAC CAT GCC ATT GTG CAG ACC CTG GTC AAT TCT Ala Asp His Leu Asm Ser Thr Asm His Ala Ile Val Glm Thr Leu Val Asm Ser
30	1497 1512 1527 1542
	GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC
	Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile
	1557 1572 1587
35	The ATE CITE THE CITE CAT CAG THE CAT AND GIG GIA CITE ANA ANT THE CAG GAD
33	Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu
	1602 1617 (408) 1636 1646 1656
	1602 1617 (408) 1616 1646 1656 ATG GTA GTA GAG GGA TGT GGG TGC CGC TGAGATCAGG CAGTCCTTGA GGATAGACAG
40	MET Val Val Glu Gly Cys Gly Cys Arg
40	
45	
	1666 1676 1686 1696 1706
	ATATACACAC CACACACA CACCACATAC ACCACACAC CACGTTCCCA
50	1716 1726 1736 1746 1756
	TCCACTCACC CACACACTAC ACAGACTGCT TCCTTATAGC TGGACTTTTA
	1766
	1766 1776 1786 1796 1806 TTTAAAAAAA AAAAAAAAA AATGGAAAAA ATCCCTAAAC ATTCACCTTG

	1816	1826	1836	1846	1856
	ACCTTATTTA	TGACTTTACG	TGCAAATGTT	TTGACCATAT	TGATCATATA
5	1866	1876	1886	1896	1906
	TTTTGACAAA	ATATATTTAT	AACTACGTAT	TAAAAGAAAA	AAATAAAATG
10	1916 AGTCATTATT	1926 TTAAAAAAAA	1936 AAAAAAAACT	1946 CTAGAGTCGA	CGGAATTC

wobei das Verfahren die nachfolgenden Schritte umfaßt:

- (a) Absuchen einer Genbank durch Hybridisieren mit einem markierten bBMP-2-Fragment, wobei die Genbank aus einer von U-2 OS abgeleiteten DNA oder cDNA konstruiert war,
- (b) Isolieren positiver Clone und

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- (c) Isolieren der DNA-Insertionen aus diesen Clonen.
- 20 12. Verfahren nach Anspruch 11, dadurch gekennzeichnet, daß das Gen menschliches BMP-4 codiert, das die in Anspruch 11 angegebene Aminosäuresequenz aufweist.
  - 13. Verfahren zur Herstellung eines Genes, das ein Protein codiert, das Eigenschaften von BMP-4 zeigt, und eine DNA-Sequenz umfaßt, die:
    - (a) sich in der Codonsequenz infolge der Degeneriertheit des genetischen Codes von einer DNA-Sequenz nach Anspruch 11 unterscheidet;
    - (b) mit einer DNA-Sequenz nach Anspruch 11 oder vorstehendem Absatz (a) hybridisiert; oder
    - (c) ein Fragment,-eine allelische-oder-eine andere Variation einer DNA-Sequenz nach Anspruch 11 darstellt, - unabhängig davon, ob die Variation zu Änderungen in der Peptidsequenz führt oder nicht,

wobei das Verfahren Standardtechniken der Molekularbiologie umfaßt.

- 14. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine genomische DNA-Sequenz ist.
- 15. Verfahren nach Anspruch 13, dadurch gekennzeichnet, daß die DNA-Sequenz eine cDNA-Sequenz ist.
- 40 16. Vektor, enthaltend das Gen oder die DNA-Sequenz nach einem der Ansprüche 1 bis 15 in einer funktionellen Verbindung mit einer Expressions-Kontrollsequenz.
  - 17. Zelle, dadurch gekennzeichnet, daß sie mit einem Vektor nach Anspruch 16 transformiert ist.
- 18. Zelle nach Anspruch 17, dadurch gekennzeichnet, daß sie eine Säugerzelle, eine Bakterienzelle, eine Insektenzelle oder eine Hefezelle ist.
  - 19. Zelle nach Anspruch 18, dadurch gekennzeichnet, daß sie eine CHO-Zelle ist.
- 20. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 zeigt, umfassend die Schritte
  - Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 1 bis 10 hergestellt wurden, und
  - Gewinnen des Proteins aus dem Kulturmedium.
  - 21. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-4 zeigt, umfassend die Schritte

- Züchten einer mit einem Expressionsvektor transformierten Zelle in einem geeigneten Kulturmedium, wobei der Expressionsvektor ein Gen oder eine DNA-Sequenz umfaßt, die nach einem der Ansprüche 11 bis 15 hergestellt wurden, und
- Gewinnen des Proteins aus dem Kulturmedium.
  - 22. Verfahren zur Herstellung eines Proteins, das Eigenschaften von BMP-2 oder BMP-4 zeigt, umfassend die Schritte
    - Züchten der Zelle nach Anspruch 17 in einem geeigneten Kulturmedium und
    - Isolieren des Proteins aus dem Kulturmedium.
  - 23. Verfahren zur Herstellung eines Arzneimittels, dadurch gekennzeichnet, daß es ein Kombinieren der nach einem der Ansprüche 20 bis 22 hergestellten Proteine, einzeln oder in Kombination, mit einem pharmakologisch verträglichen Träger umfaßt.
  - 24. Verfahren nach Anspruch 23, dadurch gekennzeichnet, daß das Arzneimittel femer eine Matrix umfaßt, die fähig ist, das Arzneimittel an die Stelle des Knochen- oder Knorpelschadens zu liefern und eine Struktur zur Induktion der Knochen- oder Knorpelbildung bereitzustellen.
  - 25. Verfahren nach Anspruch 24, dadurch gekennzeichnet, daß die Matrix Hydroxyapatit, Kollagen, Polyessigsäure oder Tricalciumphosphat umfaßt.
- 26. Verwendung eines Proteins nach einem der Ansprüche 20 bis 22, einzeln oder in Kombination, zur Herstellung eines Arzneimittels zur Induktion der Knochen- oder Knorpelbildung.

#### Revendications

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Revendications pour les Etats contractants suivants : BE, CH, DE, FR, GB, IT, LI, LU, NL, SE

1. Gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

5	eich		10 TA (	eyci.		20 GT C	AGCA		o G CII	GGGG	.40 ACIT		GAAC	50 TTG			60 AT 2	ACII	70 CCCCA
	œ==		80 TG (	œœ		90 Œ I	TIGO	10 \$\$\$\$\$\$		GAGC		TTO		120 ICT		1.			140 ŒŒ
10	ACTO		50 GC (	cric		60 AC A	CICA		0 C TG	Πœ	180 CAGC			190 GAG			00 35 (	cccc	CYCCC 510
15	GGGAG		20 GA (	GGAG		30 AG A	yyyc	24) Gaac		CATI	250 CGT			260 CCA	GGIC		70 ⁽ =A C		280 GITTT
20	TCCAT		9,0 GA (	ŒŒ	3( 3(		ICŻ)		o r co		3:20 GTGC			330 200		_	10 TC I		:350 TODAA
25	ಯನ		G G			S A					ea G				TT C eu Pi	x c			
	CTC C														AGG				
<b>30</b>	460 CCC G A <u>l</u> a A																		
35	TIC G Phe G	-C 1										AAA							
40	ASG G ATG A																		
	cas c cln P																	, ecc	
45	AAC A Asn T																		
50																			

GAG TIT ATC ACC TCA GCA GAG CIT CAG GIT TIC CCA GIU Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg GIT TA GGA AAC AAT AGC AGT TTC CAT CAC CCA ATT AAT Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ser GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGT Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Ser GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp 1000 1015 1030 CGG TGG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCG TGG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCG TCG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCGA TCGA	Ser Ser Ile Pro Thr Glu  820  835  GAA CAG ATG CAA GAT GCT Glu Gln MET Gln Asp Ala  880  ATT TAT GAA ATC ATA AAA  Ile Tyr Glu Ile Ile Lys  940  CTT TTG GAC ACC AGG TTG  Ieu Ieu Asp Thr Arg Leu  985  GTC ACC CCC GCT GTG ATG
GAG TIT ATC ACC TCA GCA GAG CIT CAG GIT TIC CCA GIU Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg Glu Phe Ile Thr Ser Ala Glu Leu Gln Val Phe Arg GIT TA GGA AAC AAT AGC AGT TTC CAT CAC CCA ATT AAT Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn Ser GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGT Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser Ser GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp 1000 1015 1030 CGG TGG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCG TGG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCG TCG ACT GCA CAG GGA CAC CCC AAC CAT GGA TTC ATC TCGA TCGA	CAA CAG ATG CAA GAT GCT Glu Gln MET Gln Asp Ala  880 ATT TAT GAA ATC ATA AAA Ile Tyr Glu Ile Ile Iys  CTT TTG GAC ACC AGG TTG Ieu Ieu Asp Thr Arg Ieu  985 GTC ACC CCC GCT GTG ATG
TTA GGA AAC AAT AGC AGT TTC CAT CAC CGA ATT AAT Leu Gly Asn Asn Ser Ser Phe His His Arg Ile Asn 15  895  910  925  CCT GCA ACA GCC AAC TCG AAA TTC CCC GTG ACC AGT Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser 19  955  970  GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp 1000  1000  1015  1030  CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC ARG TGG TGG ACT GCA TGC ATG GAA TTC ARG TGG GAA CAC CCC AAC CAT GGA TTC ARG TGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC ARG TGGA TTC ARG TGGA CAC GCC AAC CAT GGA TTC ARG TGGA CAC GCC AAC GCC	ATT TAT GAA ATC ATA AAA Ile Tyr Glu Ile Ile Iys 940 CTT TTG GAC ACC AGG TTG Ieu Ieu Asp Thr Arg Ieu 985 GTC ACC CCC GTG ATG
20 CT GCA ACA GCC AAC TOG AAA TTC CCC GTG ACC AGT Pro Ala Thr Ala Asn Ser Lys Phe Pro Val Thr Ser 1955 970  GTG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp 1000 1015 1030  GG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC ART TTC TTC TTC Ala Gln Glv His Ala Asn His Glv Phe 1000	CIT TIG GAC ACC AGG TIG Leu Leu Asp Thr Arg Leu 985 GIC ACC CCC GIG ATG
GIG AAT CAG AAT GCA AGC AGG TGG GAA AGT TTT GAT Val Asn Gln Asn Ala Ser Arg Trp Glu Ser Phe Asp  1000 1015 1030  GG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC	GTC ACC CCC GCT GTG ATG
CGG TGG ACT GCA CAG GGA CAC GCC AAC CAT GGA TTC	
25 And his till the one of the Asia has one the	1045 GTG GTG GAA GTG GCC CAC Val Val Glu Val Ala His
TIG GAG GAG AAA CAA GGT GTC TCC AAG AGA CAT GIT Leu Glu Glu Lys Gln Gly Val Ser Lys Arg His Val	11050 1105 AGG ATA AGC AGG TCT TTG Arg Ile Ser Arg Ser Leu
1120 1135	1150
CAC CAA GAT GAA CAC AGC TGG TCA CAG ATA AGG CCA His Gln Asp Glu His Ser Trp Ser Gln Ile Arg Pro	Leu Leu Val Thr Fine Gly
25 1165 1180 1195 CAT GAT GGA AAA GGG CAT COT CTC CAC AAA AGA GAA His Asp Gly Lys Gly His Pro Leu His Lys Arg Glu	1210 AAA CCT CAA GCC AAA CAC Lys Arg Gln Ala Lys His
1225 1240  AAA CAG CCG AAA CCC CTT AAG TCC AGC TGT AAG AGA Lys Gln Arg Lys Arg Leu Lys Ser Ser Cys Lys Arg	1255 CAC CCT TTG TAC GTG GAC His Pro Leu Tyr Val Ass
1270 1285 1300 TTC AGT GAC GTG GGG TGG AAT GAC TGG ATT GTG GCT The Ser Asp Val Gly Trp Asn Asp Trp Ile Val Ala	1315 CCC CCG GGG TAT CAC GCC Pro Pro Gly Tyr His Ala
1330 1345 1 TIT TAC TGC CAC GGA GAA TGC GCT TIT GCT CTG GCT The Tyr Cys His Gly Glu Cys Pro The Pro Leu Als	1350 1375 CAT CAT CTG AAC TCC ACT Asp His Leu Asn Ser Thi
sie lyt Cys ars Gly Giù Cys 275 286 215 fau 212	

•		1435 1450 1465 1480 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu
		1495 1510 1525 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC ATG GIT GIG GAG GGI TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly
10		1540(396) 1553 1563 1573 1583 1593 1603 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATTTTAG AAAAAAGAAA Cys Arg
15		AAAA
	2.	Gène codant pour la BMP-2 humaine comportant la séquence d'acides aminés donnée à la revendication 1.
20	3.	Gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN :
		(a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence de codons du fait de la dégénérescence du code génétique ;
25		(b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou (c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non.
	4.	Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADN génomique.
30	5.	Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADNc.
30		Séquence d'ADN suivant la revendication 3, qui est une séquence d'ADNc.  Gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante :
	5.	<del>and</del> the second of the second
30 35	5.	<del>and</del> the second of the second
	5.	<del>and</del> the second of the second
	5.	<del>and</del> the second of the second
35	5.	<del>and</del> the second of the second
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35	5.	<del>and</del> the second of the second
35	5.	<del>and</del> the second of the second
35 40 45	5.	<del>and</del> the second of the second

5	(1) GGC G	CAC	GAT	GGG G	15 AAA K	GGA	CAC H	CCT P	CTC L	30 CAC H	AGA R	AGA R	GAA E	ддс К	45 CGG R
10	CAA Q	GCA A	AAA K	CAC H	60 AAA K	CAG Q	CGG R	AAA K	CGC R	75 CTC L	AAG	TCC S		TGT C	90 AAG K
	AGA R	CAC H	CCT P	TTA L	105 TAT Y	GTG V	GAC D	TTC F	AGT S	120 GAT D	GTG V	GGG G			135 GAC D
15	TGG W	ATC I	GTT V	GCA A	150 CCG P	CCG P	GGG G	TAT Y	CAT H	165 GCC A	TTT F	TAC Y	TGC C		180 GGG G
20	GÁG E	TGC	CCT P	TTT F	195 CCC P	CTG L	GCC A	GAT D	CAC H	210 CTT L	AAC N	TCC S		AAT N	225 CAT H
es. 25	GCC A	ATT	CTC V	CAA Q	240 ACT T	CTG	GTC V	AAC N	TCA S	255 GTT V	AAC	TCT S		ATT I	270 CCC P
30	AAG K	•	TGC C		GTC	CCA	ACA	GAG	CTC L	AGC	GCC	ATC	TCC	ATG M	
	TAC Y	CTT L	GAT D	GAG E	330 AAT N	GAG	AAG K	GTG V	GTA V	345 TTA L	AAG K	AAC N	TAT Y	CAG O	360 D
35	ATG M	GTT V	GTC V	GAG E	GGT	TGT	GGG	TGT C	(129 CGT R	) TAGO	CACAC	197 SCA #	LAATA	4 0 LAAAT	7 'A
40	TAA		17 TA 1	TATAT	42 ATAT	7 'A TI	'AGAA	437 AAAC	AGC	SAAA:	447 AAA	TCAA	.GTTG	57 3AC	
	ACTI	4 TAAT	67 AT I	TCCC	47 AATG	7 A AG	ACTI	487 TATT	TAI	'GGAA	497 TGG	AATG	5 GAGA	107 AA	
45	AAGA		17 .CA C			7 T GA	AAAC	537 TATA	TTT	'ATAT	547 CTA	CCGA	5 AAAG	57 AA	
50	GTT		67 AA C		57 ATTT	7 T AA	TCAG	587 AGAA	TTA	TT					

- 7. Gène codant pour la BMP-2 bovine contenant la séquence d'acides aminés de la revendication 6.
- 55 8. Gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :
  - (a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ;

(b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe (a) ci-dessus ; ou (c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication 7, que ces variations résultent de changements dans la séquence peptidique ou non.

_	_	TABLE 1 A BANK A
5	9.	Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADN génomique.

10. Séquence d'ADN suivant la revendication 8, qui est une séquence d'ADNc.

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11. Gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :

10	10_	20_	30_	40	50	CCCCFFFCLY	70
	CTCTAGAGGG	<i>CAEXEGAGEA</i>	GGGAGGGAGG	GAAGGAGCGC	GGAGCCCGC	60	GGIGAGIGIG
15	80 CCATCCGAGC	90 TGAGGGAGC	100 CAGOCIGAGA	110 CCCCCCT	120 GCTCCGGCTG		140 TIGICICCCC
20	150	160	170	180	190	200	210
	GATGGGATTC	COGTOCAAGC	TATCTOGAGE	CTGCAGGGG	ACAGTCCCCG	GCCCTCGCCC	AGGITCACIG
	220	230	240	250	260	270	280
	CAACCETTCA	CACTIONNA	GCACCTGCTG	CTGGGGAGCC	CECTACTECA	GGGACCTATG	GAGCCATICC
25	290 GIAGIGŒAT	300	310 GCACIGCIGC	320 ACCITOCCIG	330 AGCCTTTCCA		350 TCAAGATTGG
<b>30</b>	360 CTGTCAAGAA	JI THOUGHT !	TIALLALA	· LLLLULLLL	400 TGTCAAGACA	CC ALC ILL	CCT Pro

		417					432					447					462	
	GGT	AAC	$\alpha$	ATG	CIG	ATG	GIC	GIT	TTA	TTA	TGC	CAA	GTC	CIG	CIA	GGA	CCC	CCC
	Gly	yzu	Arg	MET	Leu	MET	Val	Val	Leu	Leu	Cys	Gln	Val	Leu	Leu	Gly	Gly	Ala
5							•									•		
				477					492					507				
	AGC	CAI	GCT	AGT	TTG	ATA	$\alpha$	GAG	ACG	GGG	AAG	AAA	AAA	GTC	ccc	CAG	TTA	CAG
						Ile												
										-	-	•	•					
10	522					537					552					567		
	GGC	CAC	CCG	CC3	CGA	$\alpha$ c	œc	TCA	GGG	CAG		CAT	GAG	CTC	CTG	ŒG	GAC	TTC
						Arg												
	-								1									
			582					597					612					627
15	GAG	CCC		CIT	CTG	CAG	ATG		CCC	CTG	CGC	CCC.		ന്നു	CAG	CT	AGC	AAG
15						Gln												
										عميي	9	9						
					642					657					672			
	AGT	ccc	CITY:	יוידב		GAC	ب دس	אשר	~~		بينين	TIN C	~~	ىسى		ш-т	CCC	CZC
20	عصد	wa	٧	TTE	PIO	<u>az</u> A	TAL	MET	wid	تتجد	ren	Tyr	Arg	Leu	GILI	261	GIY	GIU
		687					700					~~~					777	
	Cic		~~~	C) C			702					717		~~	C) C	~~	732	~~
						ATC												
	GIU	GIU	GIU	GIU	Gin	Ile	HIS	Ser	Thr	GTA	Leu	Glu	Tyr	510	GIU	Arg	PLO	ALZ
25										•								
				747					762					777				
	AGC	œ	GCC			GIG	acc	300			CAC	CZZ	CZZ		CTG	GAG	አልሮ	ልጥሮ
	Ser	Aru	Ala	Asn	Thr	Val	Αππ	Sor	Dhe	. Wic	Hic	Glu	Glu	Hic	Ten	Glu	250	Tle
						•	AL 9	عاد	FI:E	حسنة	حلدا	GIU	GIU	حبند	-1,2:4	-014	7.31.1	446
30	792		•			807					822					837		
30			Δ~	رات) لا	(23)	AAC	4444	- CCT	mm	~			mm	220	CTC.		»CC	3000
	Pm	Glv	44.00 41.00	Son	مصحت	Asn	101	. 21-	111	7	710	Tou	111	VVC	Tou	200	70°	Tla
		<b>-</b> 1		361	GIU	ابحم	ser	Mia	Pile	Ary	File	TEU	Pile	WZII	Leu	Ser	Ser	116
			852					867	•				000					897
	יויים	מש			CH:	ATC	<b>~~</b>			C) C	CT THE	~~	882	m	~~	~~	CIC	
35	Pm	Glu	Acr.	Clu	17-1	TIC	200	. 101	31-	ىلىن دەرە	CLI	7	CIU	110	3	C3	C1-	47-1
		ara.	1721	GIU	vai	Ile	Ser	ser	Ата	G±U	LEU	Arg	Ten	me	Arg	GIU	GIII	٧ڪـــ
					010										040			
	GAC	CAC.		~	912		<b>~</b>	300		927					942		~~~	~~~
	722	Cla		Δ-1	GAT	TGG	CAA	فخلا	الغنا	TTC	CAC	ŒI.	ATA	AAC	AIT	TAT.	والاحا	GIT
40	ಬಾರಿ	GILL	GTA	PIO	WZD	dīL	GIU	Arg	GIY	Phe	His	Arg	lle	ASN	TTE	JÄL	GIU	Val
		057																
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	WIG	eleke Tamb	The same	CCA.	GCA	GAA	GIG	GIG	$\infty$	, eee	CYC	CIC	ATC	ACA	CEY.	CTA	CIG	GAC
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5	Arg :	ICG Ser	TTA Leu	CCT Pro	CFY CFY	cŋλ Ͼ	AGT Ser	GGG Gly	AAT Asn	dt. Lee	ccc Ala	elv Cyc	CIC Leu	OCC Arg	CCC Pro	CTC Leu	CTG Leu	OIC Val	
3	ACC : Thr :	227 TTT Phe	GGC Gly	CAT His	GAT Asp	ဇငေ	1242 CGG Arg	GGC Gly	CAT His	GCC Ala	TIG	1257 ACC Thir	CGA Arg	OGC Arg	CGG Arg	AGG	272 GCC Ala	AAG Lys	
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12. Gène codant pour la BMP-4 humaine comportant la séquence d'acides aminés donnée à la revendication 11.

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13. Gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN :

- (a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique;
- (b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe (a) ci-dessus ; ou
- (c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non.
- Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADN génomique.
- 15. Séquence d'ADN suivant la revendication 13, qui est une séquence d'ADNc.
- 16. Vecteur contenant le gène ou la séquence d'ADN suivant l'une quelconque des revendications 1 à 15, en association active avec une séquence de contrôle d'expression.
- 17. Cellule transformée avec un vecteur de la revendication 16.

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- 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.
- 19. Cellule suivant la revendication 18, qui est une cellule CHO.
- 20. Protéine montrant des propriétés de la BMP-2, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10.
- 21. Protéine montrant des propriétés de la BMP-2, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 1 à 10, et de récupération de ladite protéine du milieu de culture précité.
  - 22. Protéine montrant des propriétés de la BMP-4, qui est codée par un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15.
  - 23. Protéine-montrant des propriétés de la BMP-4, qui est obtenable par les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN de l'une quelconque des revendications 11 à 15, et de récupération de ladite protéine du milieu de culture précité.
- 24. Procédé de production de la protéine suivant l'une ou l'autre des revendications 21 et 23, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.
  - 25. Composition pharmaceutique comprenant les protéines de l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, et un véhicule pharmaceutiquement acceptable.
  - 26. Composition pharmaceutique suivant la revendication 25, comprenant de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et formant une structure pour induire une formation osseuse ou cartilagineuse.
  - 27. Composition pharmaceutique suivant la revendication 26, dans laquelle ladite matrice comprend de l'hydroxyapatite, du collagène, de l'acide polylactique ou du phosphate tricalcique.
- 28. Utilisation d'une protéine suivant l'une quelconque des revendications 20 à 23, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

#### Revendications pour l'Etat contractant suivant : AT

Procédé de préparation d'un gène codant pour la BMP-2 humaine comprenant la séquence d'ADN suivante :

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50	Asn	His	Ala	Ile	Val	G <u>l</u> n	Thr	Leu	Val	ريج	Ser	Val	Asn	Ser	Lvs	Ile	Pro	Lys
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1435 1450 1465 1480 GCA TGC TGT GTC CCG ACA GAA CTC AGT GCT ATC TCG ATG CTG TAC CTT GAC GAG Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile Ser MET Leu Tyr Leu Asp Glu 5 1495 1525 1510 AAT GAA AAG GIT GIA TIA AAG AAC TAT CAG GAC AIG GIT GIG GAG GGI TGT GGG Asn Glu Lys Val Val Leu Lys Asn Tyr Gln Asp MET Val Val Glu Gly Cys Gly 10 1540 (396) 1553 1563 1583 1593 1603 1573 TGT CGC TAGTACAGCA AAATTAAATA CATAAATATA TATATATATA TATATITTAG AAAAAAGAAA Cys Arq 15 AAAA , dans lequel ledit procédé comprend les étapes suivantes a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de U-2 OS avec 20 un fragment de bBMP-2 marqué par hybridation, b) l'isolement des clones positifs, et c) l'isolement des inserts d'ADN de ces clones. 2. Procédé suivant la revendication 1, dans lequel le gène code pour la BMP-2 humaine ayant la séquence d'acides 25 aminés donnée à la revendication 1. 3. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 humaine et comprenant une séquence d'ADN: 30 a) qui diffère d'une séquence d'ADN de la revendication 1 dans la séquence des codons du fait de la dégé-- nérescence-du code génétique ; ----b) qui s'hybride avec une séquence d'ADN de la revendication 1 ou du paragraphe (a) ci-dessus ; ou c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 1, que cette variation résulte de changements dans la séquence peptidique ou non, 35 dans lequel le procédé susdit comprend des techniques standards de biologie moléculaire. 4. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADN génomique. 40 5. Procédé suivant la revendication 3, dans lequel la séquence d'ADN est une séquence d'ADNc. 6. Procédé de préparation d'un gène codant pour la BMP-2 bovine comprenant la séquence d'ADN suivante : 45 50 55

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         GTTGGGAAAA CAAATATTTT AATCAGAGAA TTATT,
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dans lequel ledit procédé comprend les étapes suivantes :

a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant de foie bovin avec une sonde marquée conçue sur la base de la séquence d'acides aminés d'un fragment de bBMP-2,

b) l'isolement des clones positifs, et

c) l'isolement des inserts d'ADN de ces clones.

^{7.} Procédé suivant la revendication 6, dans lequel le gène code pour de la BMP-2 bovine ayant la séquence d'acides

aminés de la revendication 6.

		animos de la terentidadien e.
5	8.	Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-2 bovine et comprenant des séquences d'ADN :
•		a) qui diffèrent d'une séquence d'ADN de la revendication 7 dans la séquence des codons du fait de la dégénérescence du code génétique ; b) qui s'hybrident avec une séquence d'ADN de la revendication 7 ou du paragraphe a) ci-dessus ; ou
10		<ul> <li>c) représentent des fragments, des variations alléliques ou autres d'une séquence d'ADN de la revendication</li> <li>7, que ces variations résultent de changements dans la séquence peptidique ou non,</li> </ul>
		dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.
15	9.	Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
	10.	Procédé suivant la revendication 8, dans lequel la séquence d'ADN est une séquence d'ADNc.
	11.	Procédé de préparation d'un gène codant pour la BMP-4 humaine comprenant la séquence d'ADN suivante :
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	AGC Ser	CAT His	GCT Ala	477 AGT Ser	TTG Læ1	ATA Ile	CCT Pro	GJ <i>n</i> G⁄C	492 ACG Thr	GGG GLy	AAC Lys	: AAA : Lys	AAA Lys	507 GTC Val	GCC	GJ <i>n</i> Gyg	ATT Ile	C)C	
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GTC AAT TOO AGT ATC COO AAA GOO TGT TGT GTG COO ACT GAA CTG AGT GOO ATC Val Asn Ser Ser Ile Pro Lys Ala Cys Cys Val Pro Thr Glu Leu Ser Ala Ile TOO ATG CTG TAC CTG GAT GAG TAT GAT AAG GTG GTA CTG AAA AAT TAT CAG GAG Ser MET Leu Tyr Leu Asp Glu Tyr Asp Lys Val Val Leu Lys Asn Tyr Gln Glu (408)ATG GTA GTA GAG GGA TGT GGG TGC GGC TGAGATCAGG CAGTCCTTGA GGATAGACAG MET Val Val Glu Gly Cys Gly Cys Arg ATTCACCTIC ACCITATINA TEACTITACG TECAAATGIT TICACCATAT TEATCATATA TITTEACAAA : ATATATTAT AACIACGIAT TAAAAGAAA AAATAAAAG AGTCATTATT TIAAAAAAAA AAAAAAACT CIACAGIOGA CGCAATIC, dans lequel le procédé précité comprend les étapes suivantes : a) la sélection d'une bibliothèque de gènes construite à partir d'ADN ou d'ADNc provenant d'U-2 OS avec un fragment bBMP-2 marqué par hybridation, b) l'isolement des clones positifs, et c) l'isolement des inserts d'ADN de ces clones. 12. Procédé suivant la revendication 11, dans lequel le gène code pour la BMP-4 humaine ayant la séquence d'acides aminés donnée à la revendication 11. 13. Procédé de préparation d'un gène codant pour une protéine montrant des propriétés de la BMP-4 et comprenant une séquence d'ADN: 

a) qui diffère d'une séquence d'ADN de la revendication 11 dans la séquence des codons du fait de la dégénérescence du code génétique;

- b) qui s'hybride avec une séquence d'ADN de la revendication 11 ou du paragraphe a) ci-dessus ; ou
- c) représente un fragment, une variation allélique ou autre d'une séquence d'ADN de la revendication 11, que cette variation résulte de changements dans la séquence peptidique ou non,

dans lequel le procédé précité comprend des techniques standards de biologie moléculaire.

- 14. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADN génomique.
- 15. Procédé suivant la revendication 13, dans lequel la séquence d'ADN est une séquence d'ADNc.
- 16. Vecteur contenant le gène ou la séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 15, en

association active avec une séquence de contrôle d'expression.

- 17. Cellule transformée avec un vecteur de la revendication 16.
- 5 18. Cellule suivant la revendication 17, qui est une cellule mammifère, une cellule bactérienne, une cellule d'insecte ou une cellule de levure.
  - 19. Cellule suivant la revendication 18, qui est une cellule CHO.

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- 10 20. Procédé de préparation d'une protéine montrant des propriétés de la BMP-2, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 1 à 10 et de récupération de ladite protéine du milieu de culture précité.
- 15 21. Procédé de préparation d'une protéine montrant des propriétés de la BMP-4, dans lequel ledit procédé comprend les étapes de culture dans un milieu de culture approprié d'une cellule transformée avec un vecteur d'expression comprenant un gène ou une séquence d'ADN préparé suivant l'une quelconque des revendications 11 à 15 et de récupération de ladite protéine du milieu de culture précité.
- 20 22. Procédé de production d'une protéine montrant des propriétés de la BMP-2 ou BMP-4, comprenant les étapes de culture dans un milieu de culture approprié de la cellule de la revendication 17 et d'isolement de ladite protéine du milieu de culture précité.
- 23. Procédé de préparation d'une composition pharmaceutique comprenant la combinaison des protéines préparées 25 suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison avec un véhicule pharmaceutiquement acceptable.
  - 24. Procédé suivant la revendication 23, dans lequel la composition pharmaceutique susdite comprend de plus une matrice pouvant distribuer la composition au site de l'anomalie osseuse ou cartilagineuse et constituer une structure pour induire une formation osseuse ou cartilagineuse.
  - 25. Procédé suivant la revendication 24, dans leguel la matrice comprend de l'hydroxyapatite, du collagene, de l'acide polylactique ou du phosphate tricalcique.
- 35 26. Utilisation d'une protéine préparée suivant l'une quelconque des revendications 20 à 22, individuellement ou en combinaison, pour la préparation d'une composition pharmaceutique pour induire une formation osseuse ou cartilagineuse.

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